Treatment of HIV associated neurocognitive disorders

Eric Hermann Decloedt

MBChB, BSc Pharmacology (Hons), MMed (Clinical Pharmacology), FCCP (SA)

Thesis presented for the Degree of

DOCTOR OF PHILOSOPHY (PhD)

in the Department of Medicine, Division of Clinical Pharmacology,

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

June 2018

Supervisors:
Professor Gary Maartens
Professor John Joska
Dr Phumla Sinxadi

CONTENTS

DECLARATION

I, Eric Hermann Decloedt, do hereby declare that this thesis is based on 5 journal manuscripts, 4 of which have been published (chapters 2, 3, 5 and 6) and 1 is under review for publication in an international journal (chapter 4). The contents of each of these manuscripts remains unchanged from that which has been published or submitted for publication. The manuscripts are listed below with a description of my contribution to each.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication(s).

Chapter 2

Decloedt EH, Rosenkranz B, Maartens G, Joska J. Central nervous system penetration of antiretroviral drugs: pharmacokinetic, pharmacodynamic and pharmacogenomic considerations. Clin Pharmacokinet. 2015 Jun;54(6):581-98.

I wrote this invited review as background to the study in Chapter 4. We conducted a systematic review with the assistance of Cochrane South Africa who developed the search strategy to identify the 505 studies reviewed. I summarised the data and wrote the manuscript. John Joska and Gary Maartens provided input on the study selection as outlined in the chapter. My co-authors reviewed the final draft.

Chapter 3

Decloedt EH, Maartens G. Neuronal toxicity of efavirenz: a systematic review. Expert Opin Drug Saf. 2013 Nov;12(6):841-6.

This invited review was written as background to the study in Chapter 4. I summarised the data and wrote the manuscript. Gary Maartens reviewed the final draft.

Chapter 4

Pharmacogenetic and pharmacokinetic study of central nervous system penetration of efavirenz and its metabolites, tenofovir disoproxil fumarate and emtricitabine in South African patients. Decloedt EH, Sinxadi PZ, Van Zyl GU, Wiesner L, Khoo S, Joska JA, Haas DW, Maartens G. Submitted for publication.

I was the lead investigator on this clinical study and initiated the study. I lead the study design, the funding application, clinical data collection, statistical and pharmacokinetic analysis. I wrote the manuscript.

Phumla Sinxadi lead the pharmacogenetic data analysis. I worked closely with her during the analysis. She reviewed the final draft of the manuscript.

John Joska was involved in the study design, assisted with day-to-day clinical trial managment and supervised the neurocognitive assessments. He reviewed the final draft of the manuscript.

Gary Maartens and David Haas were the co-principal investigators on the study.They were involved in the study design and facilitated the pharmacokinetic and pharmacogenetic analysis. David Haas supervised the pharmacogenetic data analysis. Both reviewed the final draft of the manuscript.

Lubbe Wiesner and Saye Khoo's laboratories analysed the antiretroviral concentrations in plasma and cerebrospinal fluid as outlined in the manuscript. Both reviewed the final draft of the manuscript.

Gert van Zyl's laboratory performed the viral analysis in plasma and cerebrospinal fluid. He reviewed the final draft of the manuscript.

The following study team members in alphabetical order were also involved in the study: Laura Comrie (clinical support), Carla Freeman (clinical support), Shahieda Isaacs (viral sequencing), Pam Jordan (data capturer), Teboho Linda (neuropsychology technician). Nozipho Mawisa (study nurse), Queen Maswana (recruiter), Rasmita Ori (clinical support), Kareema Poggenpoel (administration support) and Shireen Surtie (study coordinator).

Chapter 5

Decloedt EH, Freeman C, Howells F, Casson-Crook M, Lesosky M, Koutsilieri E, Lovestone S, Maartens G, Joska JA. Moderate to severe HIV-associated neurocognitive impairment: A randomized placebo-controlled trial of lithium. Medicine (Baltimore). 2016 Nov;95(46):e5401.

I was the lead investigator on this clinical study and initiated the study. I lead the study design, the funding application, clinical data collection and statistical analysis. I wrote the manuscript.

Carla Freeman assisted me with the clinical conduct of the study. She reviewed the final manuscript.

Maia Lesosky was responsible for the randomisation and assisted with the data analysis. I worked closely with her during the analysis. She reviewed the final draft of the manuscript.

John Joska was the principal investigator and was involved in the study design, assisted with day-to-day clinical trial managment and supervised the neurocognitive assessments. He reviewed the final draft of the manuscript.

Gary Maartens was involved in the study design. He reviewed the final draft of the manuscript.

Martine Casson-Crook was responsible for the neurocognitve assessments. She reviewed the final draft of the manuscript.

Simon Lovestone and Eleni Koutsilieri were our European co-investigators. They reviewed the final draft of the manuscript.

Fleur Howells was responsible for the proton magnetic resonance spectroscopy imaging and data analysis.

The following study team members in alphabetical order were also involved in the study: Laura Comrie (clinical support), Pam Jordan (data capturer), Nicky Kramer (study pharmacist), Teboho Linda (neuropsychology technician), Queen Maswana (recruiter), Nozipho Mawisa (study nurse), Rasmita Ori (clinical support), Kareema Poggenpoel (administration support), Wynand Smythe (study pharmacist) and Shireen Surtie (study coordinator).

Chapter 6

Decloedt EH, Lesosky M, Maartens G, Joska JA. Renal safety of lithium in HIV-infected patients established on tenofovir disoproxil fumarate containing antiretroviral therapy: analysis from a randomized placebo-controlled trial. AIDS Res Ther. 2017 Feb 4;14(1):6.

This was a drug interaction sub-study from the randomised placebo-controlled trial. I lead the study design and statistical analysis. I wrote the manuscript.

Maia Lesosky assisted with the data analysis. I worked closely with her during the analysis. She reviewed the final draft of the manuscript.

John Joska and Gary Maartens were involved in the study design. They reviewed the final draft of the manuscript.

I confirm that no part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in this or any other university. I hereby grant the University of Cape Town free license to reproduce this thesis in whole or part for the purpose of research or teaching.

This thesis is presented for examination in fulfillment of the requirements for the degree of Doctor in Philosophy in Clinical Pharmacology.

Plagiarism Declaration

This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

Signed,

Signed by candidate

Eric Hermann Decloedt
11 June 2018

ABSTRACT

Title: Treatment of HIV associated neurocognitive disorders

Background

Human immunodeficiency virus (HIV) invades the central nervous system (CNS) as early as 8 days after HIV infection, causing a wide spectrum of neuropathological changes including HIV associated neurocognitive disorders (HAND). HAND is a spectrum of cognitive impairment, which in its most severe form cause marked interference with day-to-day functioning (HIV-associated dementia). Antiretroviral therapy (ART) has substantially reduced the incidence of HIV-associated dementia, but has not had an impact on the overall prevalence of HAND. The prevalence of milder stages of HAND in ART experienced individuals varies from 15 - 50%. Transporters expressed in the blood brain barrier and blood cerebrospinal fluid (CSF) barrier affect influx and efflux of drugs including antiretrovirals. Antiretrovirals that have better penetration into the CNS may result in improved cognitive function in patients with HAND, however this has not yet been conclusively shown. On the other hand, prolonged CNS exposure to high antiretroviral concentrations has been proposed as a cause of secondary decline in cognitive function as several antiretrovirals are neurotoxic. Efavirenz in particular, but also tenofovir and emtricitabine, have been shown to have direct neurotoxicity in preclinical models. Polymorphisms in genes that encode these enzymes or transporters may therefore affect antiretroviral CSF concentrations. Africans are the most genetically diverse population worldwide and South Africa has the world's largest ART programme, with most of patients currently receiving efavirenz-tenofovir-emtricitabine. The impact of pharmacogenetic polymorphisms on the pharmacokinetics of efavirenz-tenofovir-emtricitabine CNS penetration are lacking. A number of adjunctive pharmacotherapies for HAND have been studied, including lithium. Multiple mechanisms have been suggested for the potential beneficial cognitive effect of lithium, including the inhibition of glycogen synthase kinase-3-

beta, which mediates inflammation signaling pathways and neuronal apoptosis. Lithium has been used in mood disorders and other neuropsychiatric conditions for more than 40 years. In addition, lithium is a low-cost drug and widely available in public service settings in low and middle-income countries. There is a need for controlled data to evaluate the efficacy of lithium as adjunctive therapy for HAND. Finally, it is unknown whether lithium causes additive nephrotoxicity in combination with tenofovir.

Methods

We conducted a 24-week randomised placebo-controlled trial of lithium as adjunctive pharmacotherapy in participants with moderate to severe HAND established on ART for at least 6 months, with suppressed viral loads. We randomised 66 participants to lithium (n=32) or placebo (n= 34). Our primary efficacy endpoint was the change in Global Deficit Score (GDS) from baseline to 24 weeks, while our secondary endpoint was the change in proton magnetic resonance spectroscopy (^{1}H-MRS) brain metabolite concentrations. We collected paired plasma-CSF samples in 47 adult participants with and without HAND treated with efavirenz-tenofovir-emtricitabine. We considered 2049 single-nucleotide polymorphisms (SNPs), including SNPs known to affect plasma efavirenz exposure, from potentially relevant genes (*ABCC5*, *ABCG2*, *ABCB1*, *SLCO2B1*, *SCLO1A2*, *ABCC4*, *CYP2B6* and *CYP2A6*) and 880 met a linkage disequilibrium pruning threshold. We investigated genetic polymorphisms associated with CSF exposure of efavirenz and its metabolites, tenofovir and emtricitabine. The secondary objective was to explore the pharmacokinetic-pharmacodynamic relationships with neurocognitive performance. Finally, we investigated the change in estimated glomerular filtration rate (eGFR) in participants who received concomitant tenofovir and lithium.

Results

The median change in GDS between baseline and week 24 for the lithium and placebo arms were -0.57 (95% confidence interval [CI] -0.77, -0.32) and -0.56 (-0.69, -0.34) respectively,

with a mean difference of -0.054 (95% CI -0.26, 0.15); p = 0.716. The improvement remained similar when analysed according to age, severity of impairment, CD4+ count, time on ART and ART regimen. Standard ^{1}H-MRS metabolite concentrations were similar between the treatment arms. The study drug was well tolerated in both study arms. There was no statistically significant difference in the reduction in eGFR or in potassium between the two arms during the 24 weeks. We identified 9 efavirenz slow, 21 intermediate, and 17 extensive metabolizers based on composite *CYP2B6* 15582/516/983 genotype. A model that included composite *CYP2B6* 15582/516/983 genotype in univariate analyses best predicted $\log_{10}$-transformed concentrations of plasma efavirenz (β=0.34, *P*=1.7 x10^{-05}), plasma 7-hydroxy-efavirenz (β=0.45, *P*=5.8 x10^{-05}), plasma 8-hydroxy-efavirenz-to-efavirenz ratio (β=-0.29, *P*=3.7 x10^{-08}) and CSF efavirenz (β=0.33, *P*=1.7 x10^{-05}). Individual polymorphisms *CYP2B6* 516G→T and 983T→C also predicted these concentrations. Lower plasma 7-hydroxy-efavirenz concentrations were independently associated with *CYP2A6* rs10853742 (β=-0.55, *P*=3.5 x10^{-05}), *ABCB1* rs115780656 (β=-0.65, *P*=4.1 x10^{-05}) and *CYP2A6* -48A→C (β=-0.59, *P*=1.0 x10^{-02}). *CYP2A6* -48A→C was also independently associated with higher CSF 8-hydroxy-efavirenz-to-efavirenz ratio (β=0.55, *P*=4.8 x10^{-02}). The *CYP2B6* rs2279345 polymorphism was associated with lower plasma 7-hydroxy-efavirenz-to-efavirenz ratio in univariate and multivariate analyses adjusting for *CYP2B6* 516G→T and 983T→C (*P*<0.05). No polymorphisms were associated with CSF-to-plasma ratios for each of the 3 drugs, plasma or CSF concentrations of 8-hydroxy-efavirenz, tenofovir or emtricitabine, or neurocognitive performance.

Conclusion

Adjunctive lithium pharmacotherapy in patients on ART with HAND was well tolerated but had no additional benefit on neurocognitive impairment. We found that 24-week treatment of HIV-infected patients with lithium and tenofovir did not result in increased nephrotoxicity. We identified novel genetic associations with plasma efavirenz, plasma 7-hydroxy-efavirenz, plasma 7-hydroxy-efavirenz-to-efavirenz ratio, plasma 8-hydroxy-efavirenz-to-efavirenz ratio,

CSF efavirenz and CSF 8-hydroxy-efavirenz-to-efavirenz ratio concentrations. No polymorphisms were associated with CSF-to-plasma ratios of efavirenz, tenofovir or emtricitabine; plasma or CSF 8-hydroxy-efavirenz, tenofovir or emtricitabine concentrations; or neurocognitive performance.

ACKNOWLEDGEMENTS

My research was supported by the South African Medical Research Council and the European and Developing Countries Clinical Trials Partnership (SP.2011.41304.065).

I thank the participants for their commitment to advance HIV treatment knowledge by actively participating in clinical trials.

I am most grateful to my mentors and supervisors Professors Gary Maartens, John Joska and Doctor Phumla Sinxadi for their guidance and encouragement. Working with you has been inspiring. Thank you for the time that you have so generously invested in me.

The Li in HAND RCT study team, whose dedication and teamwork contributed to the success of the studies. My colleague and friend, Carla Freeman who was my right hand; Shireen Surtie, our exceptional study coordinator and face of the study; Queen Maswana who ensured that we had a steady stream of participants, Nozipho Mawisa the study nurse and the caring mother figure in the team, Martine Casson-Crook who managed the neuropsychiatric assessments competently and always volunteered to cover other trial tasks when we were in need of extra hands.

A special thank you to my partner Johan for his continuous support ever since our undergraduate studies.

Eric Decloedt

11 June 2018

CHAPTER 1

Introduction

Context

Human immunodeficiency virus (HIV) central nervous system (CNS) invasion occurs as early as 8 days after HIV infection.[1] Neuropathological changes occur and macroscopic brain changes were detected in 62% of HIV-infected patients during post-mortem examinations.[2] HIV associated neurocognitive disorders (HAND) is a spectrum of cognitive impairment, which may not interfere with everyday functioning (HIV-associated asymptomatic neurocognitive impairment) or in its most severe form cause marked interference with day-to-day functioning (HIV-associated dementia).[3,4] Patients with HAND perform below the mean for norms on neuropsychological tests by at least 1 standard deviation in at least 2 cognitive domains.[3,4] Antiretroviral therapy (ART) has substantially reduced the incidence of HIV-associated dementia, but has not had an impact on the overall prevalence of HAND.[5] The milder stages of HAND remain prevalent in ART experienced individuals with prevalence rates varying from 15 - 50% in different settings.[6–12] The social and economic burden of HAND is enormous. HAND is associated with a range of impairments of daily activities, including employment, driving and medication adherence related to a combination of neurocognitive and neurological impairment.[7] HAND will continue to place an increasing burden on health resources, especially as those living with HIV age and require residential care.[13]

CNS cell damage due to HIV sustained prior to ART (legacy effect), ongoing HIV replication in the CNS, chronic neuroinflammation caused by persistent CNS immune and glial cell activation, neurodegeneration due to aging, cerebrovascular disease and antiretroviral toxicity are some of the pathological mechanisms proposed to drive ongoing cognitive impairment.[14,15] Antiretroviral CNS penetration-effectiveness (CPE) ranking have been proposed based on the chemical properties, CSF pharmacology and effectiveness in the CNS (**Table 1**).[16] Antiretrovirals with a high CNS penetration-effectiveness (CPE) rank have been proposed to penetrate the CNS better and treat ongoing residual CNS viral replication, however it has not been conclusively shown that ART regimens with high CPE result in

improved cognitive function in patients with HIV-associated neurocognitive disorder (HAND).[16,17] A South African observational study found similar cognitive outcomes irrespective of ART regimen.[18]

Table 1 CNS penetration-effectiveness (CPE) ranking

	CPE score			
Antiretroviral class	**4**	**3**	**2**	**1**
NRTI	Zidovudine	Abacavir Emtricitabine	Didanosine Lamivudine Stavudine	Tenofovir Zalcitabine
NNRTI	Nevirapine	Delavirdine Efavirenz	Etravirine	
PI	Indinavir/r	Darunavir/r Fosamprenavir/r Indinavir Lopinavir/r	Atazanavir Atazanavir/r Fosamprenavir	Nelfinavir Ritonavir Saquinavir Saquinavir/r Tipranavir/r
Entry/fusion inhibitors		Maraviroc		Enfuvirtide
Integrase strand transfer inhibitors		Raltegravir		

Table taken unchanged from a published transcript of a lecture presented by Prof Scott Letendre.[19] NRTI = nucleoside reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitors; PI = protease inhibitors; /r = /ritonavir

Preclinical data suggest that high CNS penetrating antiretrovirals may be neurotoxic.[20] A therapeutic range for antiretrovirals in the CNS has been proposed with concentrations exceeding the therapeutic range causing ongoing cognitive impairment.[19] Higher CPE regimens have paradoxically been associated with an increased risk of HIV dementia, which could be due to antiretroviral neurotoxicity.[21] Prolonged ART exposure has been proposed as a cause of secondary decline in cognitive function when antiretroviral neurotoxicity exceeds CNS viral suppression efficacy.[22] Efavirenz in particular, but also tenofovir and emtricitabine, have been linked to direct neurotoxicity in preclinical models individually and in combination (see **Chapter 3** of thesis).[20] Interrupting ART after a median of 4.5 years was associated with improved cognitive function, especially among efavirenz recipients in one study.[23] In a randomised controlled trial (RCT), patients starting efavirenz, tenofovir and emtricitabine rather than protease inhibitors or all-nucleoside reverse transcriptase inhibitor regimens had less improvement in neurocognitive function scores after 48 weeks and patients from the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) cohort who

received efavirenz performed worse in several cognitive domains compared with protease inhibitor users after more than a year of ART.[24,25] Efavirenz and emtricitabine both have above average CPE ranking and tenofovir below average.[19] However, recently it has been shown that efavirenz protein-free concentrations are similar in plasma and cerebrospinal fluid (CSF) indicating excellent CNS penetration.[26] Human genetic variants have been associated with antiretroviral pharmacokinetics and pharmacodynamics, but ART CNS-targeted strategies have not incorporated pharmacogenetic findings (see **Chapter 2** of thesis).[27] Transporters expressed in the blood brain barrier (BBB) and blood CSF barrier (BCSFB) affect influx and efflux of drugs including antiretrovirals.[28,29] Polymorphisms in genes that encode these enzymes or transporters may therefore affect efavirenz-tenofovir-emtricitabine CSF penetration. Africans comprise the most genetic diversity worldwide and South Africa has the world's largest ART programme with most patients currently receiving efavirenz-tenofovir-emtricitabine.[30,31] The impact of pharmacogenetic polymorphisms on the pharmacokinetics of efavirenz-tenofovir-emtricitabine CNS penetration are lacking (see **Chapter 4** of thesis).

ART is the most effective treatment for HAND, as prevention or to slow down progression. However a subset of patients continue to develop HAND or have HAND progression despite ART.[32] Local data from Cape Town found that while patients with severe cognitive impairment prior to ART initiation show the most cognitive improvement, 23% - 45% of patients remained cognitively impaired after 1 year on ART.[11] The pathogenic cellular mechanisms of how HIV causes ongoing neuronal injuries have been identified but adjunctive therapy to target these pathogenic pathways have not been identified.[33–35] A number of adjunctive pharmacotherapies for HAND have been studied, including lithium.[36] There is a strong rationale to further investigate lithium as adjunctive treatment for HAND (see **Chapter 5** of thesis). Lithium is well known psychotropic agent that has been used in mood disorders and other neuropsychiatric conditions for more than 40 years. In addition, lithium is a low-cost drug and widely available in public service settings in low and middle-

income countries. Two pilot studies investigating lithium in HAND have been published. Lithium improved neurocognitive impairment in one study and neuronal integrity in both studies after 10–12 weeks of use in patients established on ART.[37,38] In the first study, 6 of the 8 individuals improved sufficiently to reduce their global deficit score (GDS) from impaired to the normal range.[37] While in the second study, cognitive performance did not improve significantly after the 10-week lithium treatment, but several changes on neuroimaging suggested neurocognitive improvement.[38] However, both studies were limited by small sample sizes (n=8 and n=15 respectively), no comparator arm and short duration of lithium treatment.

Multiple mechanisms have been suggested for the beneficial cognitive effect of lithium but the best understood mechanism is the inhibition glycogen synthase kinase-3-beta (GSK-3-β), a serine-threonine protein kinase, that mediates inflammation signaling pathways and neuronal apoptosis.[39–41] HIV-infected brain macrophages, microglia and astrocytes release neurotoxic viral proteins (Tat and gp120) and proinflammatory molecules (cytokines and free radicals) which alter the integrity of synaptic architecture and neuronal function and eventually leads to neuronal apoptosis.[35,42] The HIV protein Tat in particular has been implicated in the neuropathogenesis of HIV.[34] The addition of Tat to rat neurons increased GSK-3-β activity but the addition of lithium inhibited GSK-3-β and Tat-mediated neurotoxicity.[43] In HIV encephalitis murine models lithium reduced GSK-3-β activity and restored loss of synaptic density.[44] Lithium pre-treatment in mice exposed to gp120 was neuroprotective, suggesting that prophylactic treatment with lithium may prevent the progression of HAND.[45] Lithium might also have direct anti-inflammatory effects.[46]

Key research questions

This thesis identified and addressed the following key research questions:

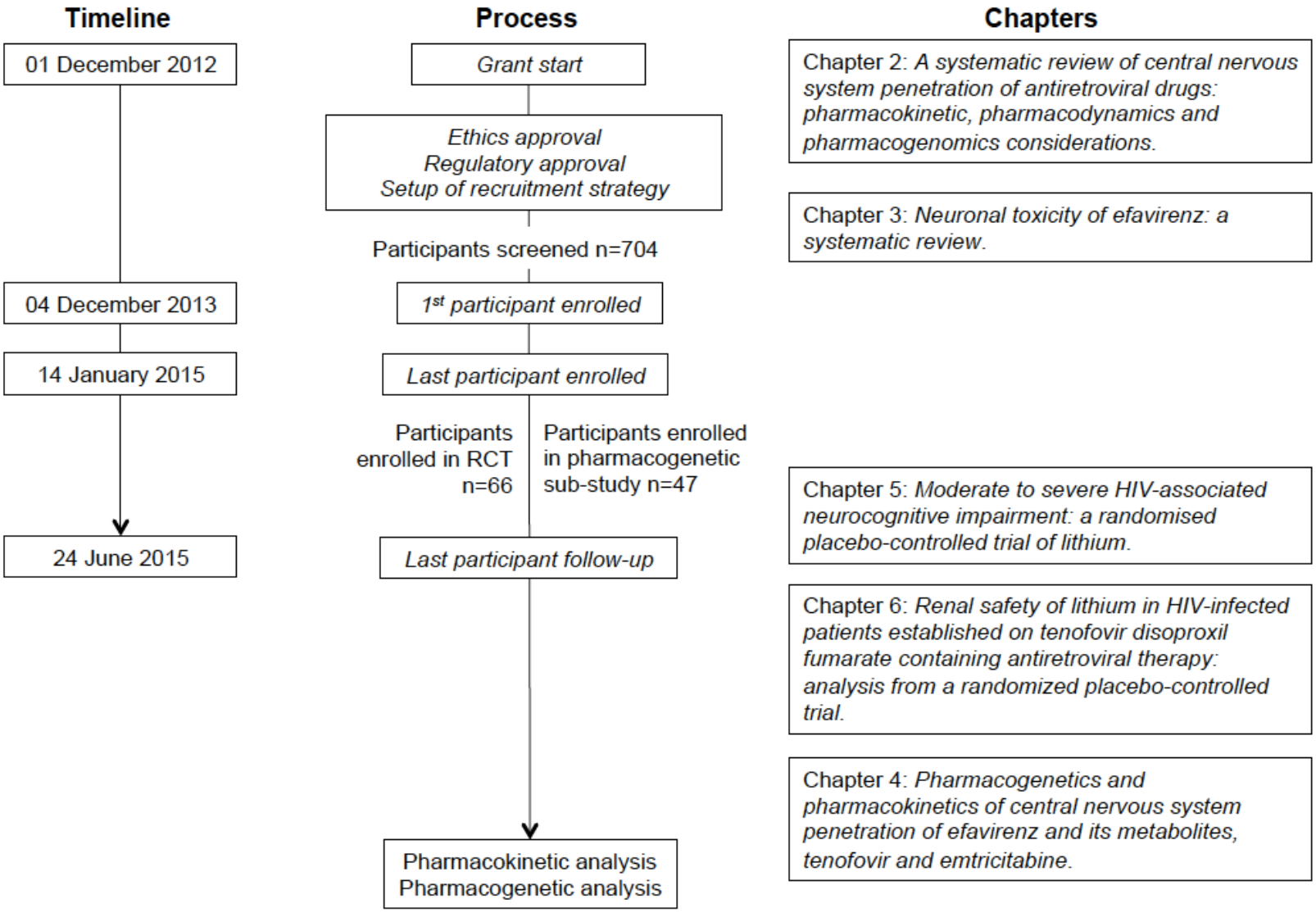
1) Which genetic polymorphisms are associated with CSF exposure of efavirenz, 8-hydroxy-efavirenz, tenofovir and emtricitabine in black South Africans?

2) Are there pharmacokinetic-pharmacodynamic relationships between CSF efavirenz, 8-hydroxy-efavirenz, tenofovir and emtricitabine exposure and neurocognitive performance?
3) Is lithium efficacious as adjunctive treatment for patients on ART with moderate to severe HAND?
4) Does lithium cause additive nephrotoxicity in combination with tenofovir?

Description of the project

We screened participants at referral sites and involved as many sites as possible in the Cape Town metropole for referral to our study site at Groote Schuur Hospital. Our most productive referral site was Nolungile Site C clinic in Khayelitsha outside of Cape Town. Participants who were screened but did not meet the cognitive impairment inclusion criteria of the RCT were invited to participate in the pharmacokinetic-pharmacogenetic sub-study. **Figure 1** outlines the chronological order of the study.

Figure 1 Outline of the chronological order of the study and the manuscript preparation and publication



Coherence of the thesis

Three key points link the coherence of this thesis. First, I am the first author and lead investigator on all the studies and manuscripts included. I initiated and lead all the projects from conception to execution to publication. Second, the work stems from a single project with the same cohort of patients. Third, the unifying theme is the pharmacological treatment of patients with HAND.

Outline of the thesis

Chapters 2 and 3 are reviews as background and literature to the thesis. Chapter 2 is a systematic review on CNS penetration of ART with a critical focus on pharmacokinetic, pharmacodynamics and pharmacogenomics considerations. In chapter 3 I review the data to support the neurotoxicity of efavirenz. Both reviews set the scene for chapter 4.

Chapter 4 reports on the pharmacogenetic sub-study with 47 HIV-infected participants established on ART with various degrees of HAND. We conducted a cross-sectional study and measured efavirenz-tenofovir-emtricitabine exposure in the CSF and plasma and assessed neurocognitive function. We investigated potential genetic polymorphisms associated with CSF exposure of efavirenz, 8-hydroxy-efavirenz, tenofovir and emtricitabine in black South Africans. The secondary objective was to explore the pharmacokinetic-pharmacodynamic relationships of CSF antiretroviral exposure with neurocognitive performance.

In **chapter 5** I describe a 24-week RCT trial of 66 participants randomised to lithium (n=32) or placebo (n=34) to assess the efficacy and safety of lithium as adjunctive pharmacotherapy in patients with moderate to severe HAND. There is no effective adjunctive therapy and lithium was an adjunctive intervention with some evidence of efficacy from pilot studies. Our primary efficacy endpoint was the change GDS from baseline to 24 week in the placebo arm compared to the lithium arm. Our secondary endpoint was the change between

baseline (-4 to 0 weeks) and week 23 in proton magnetic resonance spectroscopy brain metabolite concentrations in three brain areas.

Renal safety was a major concern when studying lithium in combination with tenofovir. Both tenofovir and lithium are associated with renal tubular toxicity, which could be additive. **Chapter 6** describes, to my knowledge, the first safety data of co-administered lithium with tenofovir. I framed the relevance of this study in the context the more common indication of lithium as an effective mood stabiliser. The prevalence of bipolar disorder in HIV-infected patients is 4 to 5 times higher than the general population and tenofovir is frequently used as part of ART.

I summarised my findings, conclusion and future research priorities in **chapter 7**.

References

1. Valcour V, Chalermchai T, Sailasuta N, et al. Central nervous system viral invasion and inflammation during acute HIV infection. *J Infect Dis*. 2012;206(2):275-282. doi:10.1093/infdis/jis326.
2. Vago L, Trabattoni G, Lechi A, Cristina S, Budka H. Neuropathology of AIDS dementia. A review after 205 post mortem examinations. *Acta Neurol (Napoli)*. 1990;12(1):32-35.
3. Antinori A, Arendt G, Becker JT, et al. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology*. 2007;69(18):1789-1799. doi:10.1212/01.WNL.0000287431.88658.8b.
4. Gisslén M, Price RW, Nilsson S. The definition of HIV-associated neurocognitive disorders: are we overestimating the real prevalence? *BMC Infect Dis*. 2011;11:356. doi:10.1186/1471-2334-11-356.
5. Heaton RK, Franklin DR, Ellis RJ, et al. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: Differences in rates, nature, and predictors. *J Neurovirol*. 2011;17(1):3-16. doi:10.1007/s13365-010-0006-1.
6. McNamara PH, Coen R, Redmond J, Doherty CP, Bergin C. A High Prevalence Rate of a Positive Screen for Cognitive Impairment in Patients With Human Immunodeficiency Virus Attending an Irish Clinic. *Open forum Infect Dis*. 2017;4(1):ofw242. doi:10.1093/ofid/ofw242.
7. Heaton RK, Clifford DB, Franklin DR, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*. 2010;75(23):2087-2096. doi:10.1212/WNL.0b013e318200d727.
8. Robertson KR, Smurzynski M, Parsons TD, et al. The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS*. 2007;21(14):1915-1921. doi:10.1097/QAD.0b013e32828e4e27.
9. Cysique LA, Brew BJ. Prevalence of non-confounded HIV-associated neurocognitive

impairment in the context of plasma HIV RNA suppression. *J Neurovirol*. 2011;17(2):176-183. doi:10.1007/s13365-011-0021-x.

10. Wright E, Brew B, Arayawichanont A, et al. Neurologic disorders are prevalent in HIV-positive outpatients in the Asia-Pacific region. *Neurology*. 2008;71(1):50-56. doi:10.1212/01.wnl.0000316390.17248.65.
11. Joska JA, Westgarth-Taylor J, Hoare J, et al. Neuropsychological outcomes in adults commencing highly active anti-retroviral treatment in South Africa: a prospective study. *BMC Infect Dis*. 2012;12(1):39. doi:10.1186/1471-2334-12-39.
12. Belete T, Medfu G, Yemiyamrew E. Prevalence of HIV Associated Neurocognitive Deficit among HIV Positive People in Ethiopia: A Cross Sectional Study at Ayder Referral Hospital. *Ethiop J Health Sci*. 2017;27(1):67-76. http://www.ncbi.nlm.nih.gov/pubmed/28458492.
13. Cysique LA, Bain MP, Brew BJ, Murray JM. The burden of HIV-associated neurocognitive impairment in Australia and its estimates for the future. *Sex Health*. 2011;8(4):541-550. doi:10.1071/SH11003.
14. Nightingale S, Winston A, Letendre S, et al. Controversies in HIV-associated neurocognitive disorders. *Lancet Neurol*. 2014;13(11):1139-1151. doi:10.1016/S1474-4422(14)70137-1.
15. Nightingale S, Winston A. Measuring and managing cognitive impairment in HIV. *Aids*. 2017;31(January):S165-S172. doi:10.1097/QAD.0000000000001402.
16. Letendre S, Marquie-Beck J, Capparelli E, et al. Validation of the CNS penetration-effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Arch Neurol*. 2008;65(1):65-70. doi:10.1001/archneurol.2007.31.
17. Ellis RJ, Letendre S, Vaida F, et al. Randomized trial of central nervous system-targeted antiretrovirals for HIV-associated neurocognitive disorder. *Clin Infect Dis*. 2014;58(7):1015-1022. doi:10.1093/cid/cit921.
18. Cross HM, Combrinck MI, Joska JA. HIV-associated neurocognitive disorders: antiretroviral regimen, central nervous system penetration effectiveness, and cognitive

outcomes. *S Afr Med J*. 2013;103(10):758-762. http://www.ncbi.nlm.nih.gov/pubmed/24079630.

19. Letendre S. Central nervous system complications in HIV disease: HIV-associated neurocognitive disorder. *Top Antivir Med*. 2011;19(4):137-142. http://www.ncbi.nlm.nih.gov/pubmed/22156215. Accessed May 7, 2014.
20. Robertson K, Liner J, Meeker RB. Antiretroviral neurotoxicity. *J Neurovirol*. 2012;18(5):388-399. doi:10.1007/s13365-012-0120-3.
21. Caniglia EC, Cain LE, Justice A, et al. Antiretroviral penetration into the CNS and incidence of AIDS-defining neurologic conditions. *Neurology*. 2014;83(2):134-141. doi:10.1212/WNL.0000000000000564.
22. Underwood J, Robertson KR, Winston A. Could antiretroviral neurotoxicity play a role in the pathogenesis of cognitive impairment in treated HIV disease?. *AIDS*. 2015;29(3):253-261. doi:10.1097/QAD.0000000000000538.
23. Robertson KR, Su Z, Margolis DM, et al. Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology*. 2010;74(16):1260-1266. doi:10.1212/WNL.0b013e3181d9ed09.
24. Winston A, Duncombe C, Li PCK, et al. Does choice of combination antiretroviral therapy (cART) alter changes in cerebral function testing after 48 weeks in treatment-naive, HIV-1-infected individuals commencing cART? A randomized, controlled study. *Clin Infect Dis*. 2010;50(6):920-929. doi:10.1086/650743.
25. Ma Q, Vaida F, Wong J, et al. Long-term efavirenz use is associated with worse neurocognitive functioning in HIV-infected patients. *J Neurovirol*. 2016;22(2):170-178. doi:10.1007/s13365-015-0382-7.
26. Avery LB, Sacktor N, McArthur JC, Hendrix CW. Protein-free efavirenz concentrations in cerebrospinal fluid and blood plasma are equivalent: applying the law of mass action to predict protein-free drug concentration. *Antimicrob Agents Chemother*. 2013;57(3):1409-1414. doi:10.1128/AAC.02329-12.
27. Haas DW, Tarr PE. Perspectives on pharmacogenomics of antiretroviral medications

and HIV-associated comorbidities. *Curr Opin HIV AIDS*. 2015;10(2):116-122. doi:10.1097/COH.0000000000000134.

28. Suhy AM, Webb A, Papp AC, Geier EG, Sadee W. Expression and splicing of ABC and SLC transporters in the human blood-brain barrier measured with RNAseq. *Eur J Pharm Sci*. 2017;103:47-51. doi:10.1016/j.ejps.2017.02.010.
29. Varatharajan L, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary of current knowledge and recommendations for further research. *Antiviral Res*. 2009;82(2):A99-109. doi:10.1016/j.antiviral.2008.12.013.
30. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. *Annu Rev Genomics Hum Genet*. 2008;9:403-433. doi:10.1146/annurev.genom.9.081307.164258.
31. World Health Organisation. Antiretroviral therapy coverage data and estimates by country. *Glob Heal Obs data Repos*. 2017. http://apps.who.int/gho/data/node.main.626?lang=en. Accessed April 23, 2018.
32. Joska J a, Gouse H, Paul RH, Stein DJ, Flisher AJ. Does highly active antiretroviral therapy improve neurocognitive function? A systematic review. *J Neurovirol*. 2010;16(2):101-114. doi:10.3109/13550281003682513.
33. Saylor D, Dickens AM, Sacktor N, et al. HIV-associated neurocognitive disorder--pathogenesis and prospects for treatment. *Nat Rev Neurol*. 2016;12(4):234-248. doi:10.1038/nrneurol.2016.27.
34. Zayyad Z, Spudich S. Neuropathogenesis of HIV: from initial neuroinvasion to HIV-associated neurocognitive disorder (HAND). *Curr HIV/AIDS Rep*. 2015;12(1):16-24. doi:10.1007/s11904-014-0255-3.
35. Jaeger LB, Nath A. Modeling HIV-associated neurocognitive disorders in mice: new approaches in the changing face of HIV neuropathogenesis. *Dis Model Mech*. 2012;5(3):313-322. doi:10.1242/dmm.008763.
36. McGuire JL, Barrett JS, Vezina HE, Spitsin S, Douglas SD. Adjuvant therapies for

HIV-associated neurocognitive disorders. *Ann Clin Transl Neurol*. 2014;1(11):938-952. doi:10.1002/acn3.131.

37. Letendre SL, Woods SP, Ellis RJ, et al. Lithium improves HIV-associated neurocognitive impairment. *AIDS*. 2006;20(14):1885-1888. doi:10.1097/01.aids.0000244208.49123.1b.

38. Schifitto G, Zhong J, Gill D, et al. Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. *J Neurovirol*. 2009;15(2):176-186. doi:10.1080/13550280902758973.

39. Crowder RJ, Freeman RS. Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. *J Neurosci*. 1998;18(8):2933-2943. http://www.ncbi.nlm.nih.gov/pubmed/9526010.

40. Lazzara CA, Kim YH. Potential application of lithium in Parkinson's and other neurodegenerative diseases. *Front Neurosci*. 2015;9(OCT). doi:10.3389/fnins.2015.00403.

41. Peineau S, Bradley C, Taghibiglou C, et al. The role of GSK-3 in synaptic plasticity. *Br J Pharmacol*. 2008;153 Suppl:S428-37. doi:10.1038/bjp.2008.2.

42. Lindl K a, Marks DR, Kolson DL, Jordan-Sciutto KL. HIV-associated neurocognitive disorder: pathogenesis and therapeutic opportunities. *J Neuroimmune Pharmacol*. 2010;5(3):294-309. doi:10.1007/s11481-010-9205-z.

43. Maggirwar SB, Tong N, Ramirez S, Gelbard HA, Dewhurst S. HIV-1 Tat-mediated activation of glycogen synthase kinase-3beta contributes to Tat-mediated neurotoxicity. *J Neurochem*. 1999;73(2):578-586. http://www.ncbi.nlm.nih.gov/pubmed/10428053.

44. Dou H, Ellison B, Bradley J, et al. Neuroprotective mechanisms of lithium in murine human immunodeficiency virus-1 encephalitis. *J Neurosci*. 2005;25(37):8375-8385. doi:10.1523/JNEUROSCI.2164-05.2005.

45. Everall IP, Bell C, Mallory M, et al. Lithium ameliorates HIV-gp120-mediated

neurotoxicity. *Mol Cell Neurosci*. 2002;21(3):493-501. http://www.ncbi.nlm.nih.gov/pubmed/12498789.

46. Basselin M, Kim H-W, Chen M, et al. Lithium modifies brain arachidonic and docosahexaenoic metabolism in rat lipopolysaccharide model of neuroinflammation. *J Lipid Res*. 2010;51(5):1049-1056. doi:10.1194/jlr.M002469.

CHAPTER 2

A systematic review of central nervous system penetration of antiretroviral drugs: pharmacokinetic, pharmacodynamics and pharmacogenomics considerations.

Central Nervous System Penetration of Antiretroviral Drugs: Pharmacokinetic, Pharmacodynamic and Pharmacogenomic Considerations

Eric H. Decloedt, Bernd Rosenkranz, Gary Maartens & John Joska

Clinical Pharmacokinetics

ISSN 0312-5963
Volume 54
Number 6

Clin Pharmacokinet (2015) 54:581-598
DOI 10.1007/s40262-015-0257-3

Springer

Clin Pharmacokinet (2015) 54:581–598
DOI 10.1007/s40262-015-0257-3

REVIEW ARTICLE

Central Nervous System Penetration of Antiretroviral Drugs: Pharmacokinetic, Pharmacodynamic and Pharmacogenomic Considerations

Eric H. Decloedt · Bernd Rosenkranz · Gary Maartens · John Joska

Published online: 17 March 2015

Abstract The prevalence of HIV-associated neurocognitive disorder (HAND) is increasing despite the widespread use of combination antiretroviral therapy (ART). Initial reports suggest that the use of antiretrovirals with good central nervous system (CNS) penetration leads to better neurocognitive outcomes, but this has not yet been confirmed in a large cohort study or randomised controlled trial. There is emerging evidence that high CNS concentrations of some antiretrovirals are potentially neurotoxic and may be associated with the development of HAND. Antiretroviral CNS exposure is ideally determined by determining the ratio of cerebrospinal fluid (CSF):plasma area under the curve of unbound drug, but usually only total drug concentrations are measured and the ratio of CSF:plasma drug concentration is done at a single time point, which can result in misclassifying CNS penetration ability. Efavirenz was previously thought to have poor CNS penetration, measured by the CSF:plasma ratio (0.87 %), but when unbound concentrations were measured it was found to have good CNS penetration (85 %). Indinavir and efavirenz are the only antiretroviral drugs for which CNS area under the concentration–time curves using unbound plasma and CSF concentrations has been calculated. Patient data to support the contribution of blood–brain barrier transporter polymorphisms to CNS antiretroviral concentrations are currently limited and lack power to detect true associations. Correlations between CNS antiretroviral exposure and effect is multifaceted, and to accurately predict CNS effects there is a need to develop a sophisticated intra-brain pharmacokinetic–pharmacodynamic–pharmacogenetic model that includes transporters as well as the influence of HIV.

Key Points

There are limited antiretroviral pharmacokinetic studies that adequately estimate CNS exposure calculating area under the concentration–time curve using total and unbound cerebrospinal fluid antiretroviral concentrations.

Data on the clinical relevance and extent of the contribution of polymorphisms in genes encoding for blood–brain transporters to CNS antiretroviral exposure are limited due to the small number of studies and lack of power.

Current understanding and categorizing of antiretroviral CNS penetration has not translated into better clinical outcomes and there is a need to develop a sophisticated intra-brain pharmacokinetic–pharmacodynamic–pharmacogenetic model that includes transporters as well as the influence of HIV.

E. H. Decloedt (✉) · B. Rosenkranz
Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Tygerberg Hospital, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa
e-mail: ericdecloedt@sun.ac.za

G. Maartens
Division of Clinical Pharmacology, Department of Medicine, Groote Schuur Hospital, University of Cape Town, Observatory 7925, South Africa

J. Joska
Division of Neuropsychiatry, Department of Psychiatry and Mental Health, Groote Schuur Hospital, University of Cape Town, Observatory 7925, South Africa

1 Introduction

The overall prevalence of all forms of HIV-associated neurocognitive disorders (HANDs) is increasing despite the widespread use of combination antiretroviral therapy (ART) [1]. While the incidence of severe disorders, such as HIV-dementia (HIV-D), has significantly reduced, milder forms of HAND are on the rise. This disease burden is, in large part, being driven by the longer life expectancy of treated individuals and the associated neurocognitive impairment due to cardiovascular disease and related degenerative diseases of aging [2]. HAND is associated with a range of functional impairments that can affect employment, driving and medication adherence [1, 3]. Proposed mechanisms of the development or progression of HAND in people receiving ART include persistent neurodegeneration and neurotoxicity from antiretroviral drugs [4]. In vitro data suggest that antiretroviral drugs cause neurotoxicity at therapeutic doses [5, 6]. Better central nervous system (CNS)-penetrating antiretroviral drugs were initially associated with better neurocognitive outcomes, but large cohort data suggest an associated increased risk of developing dementia [7, 8].

The association between viral replication and cerebrospinal fluid (CSF) antiretroviral concentrations has been the subject of intensive investigation. Physiochemical properties of the drug (size, lipophilicity, plasma protein binding, active transport into the CNS and metabolism in the CNS) can predict CNS drug exposure to some extent but pharmacokinetic studies are required for confirmation [9]. Pharmacokinetic studies of CNS penetration of drugs are usually done by sampling CSF, which is in close contact with brain extracellular fluid [10]. There are caveats when making inferences about CNS drug exposure using CSF drug concentrations [11, 12]. First, CSF acts as a slowly equilibrating compartment relative to plasma with reduced and delayed concentration peaks and an overall flatter profile shape of the area under the concentration–time curves (AUCs) [13]. CSF:plasma drug ratios, which are often used as a measure of CNS exposure, will therefore vary depending on the time of sampling. Estimation of CSF and plasma AUCs followed by calculating the ratio of exposure is a more robust method of estimating CNS drug penetration [11]; however, CSF AUC estimation is hampered by the difficulty in obtaining multiple CSF samples. Second, measuring total drug concentrations rather than unbound concentrations gives misleading information about CNS exposure as only unbound drug is able to act at the receptor site [14]. Efavirenz CSF penetration was thought to be limited based on total efavirenz concentrations, but efavirenz CSF penetration is excellent, with similar plasma and CSF unbound concentrations [14]. Third, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) are both highly protein bound, with NNRTIs predominantly binding to α1-acid glycoprotein, while PIs bind to albumin [14, 15]. Antiretroviral entry into the CNS is therefore governed largely by multiple influx and efflux drug transporters at the blood–brain barrier and the blood–CSF barrier [16–19]. Drug exchange between blood, CSF and brain extracellular fluid does not occur freely, and drug concentration measurements made in any one of these compartments may not accurately reflect events in the other compartments [10, 20]. Fourth, genetic polymorphisms in relevant metabolizing enzymes and transporters at the different blood–brain interfaces may influence drug disposition and response [21, 22]. Last, HIV disease compromises the blood–brain barrier integrity which will influence drug exposure [23]. Healthy volunteer data may therefore not reflect drug exposure seen in HIV-infected patients.

A recent review in *Clinical Pharmacokinetics* discussed the pharmacokinetics and pharmacodynamics of antiretrovirals in the CNS [24]. We critically reviewed the pharmacokinetic data of antiretroviral drug exposure in the CNS with the focus on the quality of the CSF pharmacokinetic studies according to the different antiretroviral drug classes, which included a focus on total and unbound concentration analysis. We identified variables that influence CNS exposure, including the potential role of genetic polymorphisms on drug transporters and their influence on CNS antiretroviral exposure. Finally, we explored links between antiretroviral CNS pharmacokinetics and clinical outcomes.

1.1 Study Selection

We conducted a systematic search in the PubMed database from inception until 1 January 2015. Two reviewers (ED and JJ) independently identified studies that reported on the measurement of CSF antiretroviral concentrations in HIV-infected patients. Discrepancies between the two reviewers were mediated by a third reviewer (GM). We evaluated the quality of the data using the following criteria: a priori sample size calculation, CSF and plasma antiretroviral bound and unbound drug analysis, and estimation of CSF and plasma antiretroviral exposure using AUC. We evaluated pharmacodynamic or clinical outcomes if any were reported, and excluded studies that evaluated antiretroviral drug exposure in animal models.

1.2 Search Strategy

We conducted multiple searches on human antiretroviral pharmacokinetic studies in HIV-infected patients which

measured drug concentrations in the CSF. For *search 1* we used the following Medical Subject Heading (MeSH) terms: Search (HIV Infections[MeSH] OR HIV[MeSH] OR hiv[tiab] OR hiv-1*[tiab] OR hiv-2*[tiab] OR hiv1[tiab] OR hiv2[tiab] OR hiv infect*[tiab] OR human immunodeficiency virus[tiab] OR human immunedeficiency virus[tiab] OR human immuno-deficiency virus[tiab] OR human immune-deficiency virus[tiab] OR ((human immun*[tiab]) AND (deficiency virus[tiab])) OR acquired immunodeficiency syndrome[tiab] OR acquired immunedeficiency syndrome[tiab] OR acquired immuno-deficiency syndrome[tiab] OR acquired immune-deficiency syndrome[tiab] OR ((acquired immun*[tiab]) AND (deficiency syndrome[tiab])) OR "sexually transmitted diseases, Viral"[MeSH:NoExp]). For *search 2* we used the following MeSH terms: Search (antiretroviral therapy, highly active[MeSH] OR anti-retroviral agents[MeSH] OR antiviral agents[MeSH:NoExp] OR ((anti[tiab]) AND (hiv[tiab])) OR antiretroviral*[tiab] OR ((anti[tiab]) AND (retroviral*[tiab])) OR HAART[tiab] OR ((anti[tiab]) AND (acquired immunodeficiency[tiab])) OR ((anti[tiab]) AND (acquired immuno-deficiency[tiab])) OR ((anti[tiab]) AND (acquired immune-deficiency[tiab])) OR ((anti[tiab]) AND (acquired immun*[tiab]) AND (deficiency [tiab])). For *search 3* we used the following MeSH terms: Search (central nervous system[mh] OR central nervous system*[tiab] OR cerebrospinal fluid[mh] OR cerebrospinal fluid*[tiab]). For *search 4* we used the following MeSH terms: Search (pharmacokinetics[mh] OR pharmacokinetics[tiab] OR transport*[tiab] OR penetra*[tiab] OR blood-brain barrier[mh] OR blood-brain barrier*[tiab]). We combined searches 1 and 2 and further refined the search by performing searches 3 and 4. The search strategy identified 505 articles that studied the CSF exposure of 18 different antiretroviral drugs. A meta-analysis was not possible due to study methodology heterogeneity. We opted to discuss each antiretroviral drug class critically, and conducted an additional search focused on human genetic polymorphisms and the association with CSF antiretroviral exposure.

2 Pharmacokinetics and Pharmacodynamics

Various pharmacodynamic markers for HIV CNS are used [25]. CSF inhibitory concentrations are frequently used in antiretroviral pharmacokinetic studies. Recently, CSF 95 % inhibitory quotients (IQ_{95}) were proposed as an improved marker, with high CSF IQ_{95} being associated with better CSF viral suppression and a lower prevalence of CSF escape [26]. IQ_{95} is the ratio between the CSF concentration and the 95 % inhibitory concentration (IC_{95}), and a ratio of more than 1 is considered adequate exposure. The relationship between IQ_{95} and the potential for neurotoxicity has not been investigated. Clinical neurocognitive endpoints and the relationship with antiretroviral pharmacokinetics has been best described by the CNS penetration-effectiveness (CPE) score hypothesis studies. The updated CPE score places antiretroviral drugs into four categories according to physiochemical drug properties, measured CSF drug concentrations, and efficacy as determined by CSF viral suppression and neurocognitive improvement [27]. Antiretrovirals with lower CPE scores are associated with higher CSF viral loads [8]. Antiretrovirals with higher CPE scores penetrate the CNS better and are thought to be more appropriate for patients with HIV-associated neurocognitive symptoms. In uncontrolled observational studies, higher CNS-penetrating antiretrovirals were associated with better CSF viral load suppression, while others also showed an association with improved neurocognitive outcomes compared with lower penetrating antiretrovirals [8, 28–30]. In a large cohort of nearly 62,000 patients followed-up for a median of 37 months, patients receiving drugs with a high CPE score were found to be at increased risk of developing dementia, with a hazard ratio of 1.74 (95 % confidence interval 1.15–2.65), compared with patients receiving ART with a lower CPE score [7]. Antiretroviral-mediated increase in the deposition of β-amyloid, as well as neurotoxicity, were cited as some of the reasons for the findings [6, 31]; however, this finding should be further studied as the association may have been confounded by the majority of patients switching from their original ART regimen, and initiation with a high CPE regimen may have been informed by patients presenting with neurocognitive symptoms. The association between higher CPE scores and better neurocognitive outcomes was not demonstrated in a recent randomised controlled trial, but the trial was underpowered and stopped early due to low accrual [32]. Current understanding and categorizing of antiretroviral CNS penetration has not translated into better clinical outcomes. In the following sections, we will review the pharmacokinetic data on which CNS penetration inferences are based on, and highlight the gaps in our knowledge.

2.1 Nucleoside and Nucleotide Reverse Transcriptase Inhibitors

The nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) have good CNS penetration, with the exception of tenofovir (see Table 1). Exposure of NRTIs in the CSF exceeds the in vitro inhibitory concentration to suppress 50 % viral replication (IC_{50}), but no unbound data are available (see Table 2). However, CSF sampling measures extracellular drug concentrations and NRTIs require intracellular phosphorylation to be pharmacologically active,

Table 1 Nucleoside and nucleotide reverse transcriptase inhibitors' central nervous system pharmacokinetic data

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Abacavir				
Range of 150–600 mg 12-hourly				
Total concentration CSF/plasma AUC ratio: 36 ± 5 %[b] (95 % CI 28–46)	Total concentration: 128 (37–384) ng/ml[a]	Total concentration: 139 ng/ml (<40 to 1130) ng/ml[a]	POPPK estimates of paired CSF–plasma total concentrations of 70 CSF and 64 plasma samples taken at various time points during the dosing interval	[83]
Range of 600–1800 mg daily				
Total concentration CSF/plasma AUC ratio: 35 (31–44) %[a]	Total AUC_{∞}: 5.14 (2.01–13.13) μg·h/ml[c]	Total AUC_{∞}: 12.81 (8.66–18.96) μg·h/ml[c]	Analysis of seven paired CSF–plasma total concentrations taken during the first 6 h of dosing from three patients who received a single dose	[36]
Total concentration CSF/plasma ratio: 42 (80–173) %[a]	Total concentration: 0.14 (0.09–0.19) μg/ml[a]	Total AUC_{∞}: 0.74 (0.66–0.84) μg·h/ml[c] to 0.92 (0.83–1.02) μg·h/ml, depending on the dose	Analysis of nine mid-dose paired CSF–plasma total concentrations taken 1.5 h post-dosing	[37]
Lamivudine				
Range of 8–20 mg/kg daily				
Total concentration CSF/plasma ratio: 6 (4–8) %[e]	Total concentration: range 94–328 ng/l	Not stated	Analysis of six paired CSF–plasma total concentrations taken at 2 h post-dosing from six patients	[84]
Range of 0.5–10 mg/kg/daily				
Total concentration CSF/plasma ratio: 12 (0–46) %[a]	Total concentration: 0.07 (undetectable–0.12) μM[a] to 0.99 (0.32–2.23) μM[a]	Total AUC_{oral}: 2.84 (1.04) μM·h[c] to 61.8 (35.8) μM·h[c], depending on the dose	Analysis of 68 paired CSF–plasma total concentrations taken at 2–4 h post-dosing from 44 children	[85]
150 mg 12-hourly				
Total concentration CSF/plasma ratio: median range 6–30 % (interpreted from a graph)	Total concentration: range 0.29–0.35 μmol/l	Total concentration: median range 400–960 ng/ml (interpreted from a graph)	Analysis of 22 paired CSF–plasma total concentrations taken at 2–8 h post-dosing from 22 patients	[86]
Total concentration CSF/plasma ratio: 15.1 (1.3)[c] (range 12.4–17.5) %	Total AUC_{12}: 3346 (219) nM·h[c] (range 2768–3740) nM·h	Total AUC_{12}: 22,216 (580)[c] (range 21,307–23,787) nM·h	Analysis of ultra-intensive paired CSF–plasma total concentrations taken twice over 48 h from four patients	[81]
Total concentration CSF/plasma ratio: 22.9 (0–49) %[e]	Not reported	Not reported	Analysis of 55 paired CSF–plasma total concentrations	[49]
Stavudine				
Total concentration CSF/plasma ratio: 38.9 (3.9)[c] (range 34.1–50.4) %	Total AUC_{12}: 1814 (414) nM·h[c] (range 1034–2938) nM·h	Total AUC_{12}: 4524 (622)[c] (range 3035–5825) nM·h	Analysis of ultra-intensive paired CSF–plasma total concentrations taken twice over 48 h from four patients	[81]
Total concentration CSF/plasma ratio: median range 20–85 % (interpreted from a graph)	Total concentration: range 0.20–0.27 μmol/l	Total concentration: median range 100–280 ng/ml (interpreted from a graph)	Analysis of 17 paired CSF–plasma total concentrations taken at 2–8 h post-dosing from 17 patients	[86]

Table 1 continued

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Total concentration CSF/plasma ratio: 40 (6) %[c]	Total concentration: 62.09 (12.88) ng/ml[c]	TotalAUC_{∞}: 2116 (346.52) ng·h/ml[c]	Analysis of four paired CSF–plasma total concentrations taken at 4–5 h post-dosing from four patients	[87]
Total concentration CSF/plasma ratio: 20.4 (0.0–20.4)[a]	Not reported	Not reported	Analysis of 31 paired CSF–plasma total concentrations.	[49]
Tenofovir				
Total concentration CSF/plasma ratio: 5.7 (3.0–10.0)[c] (range 0.4–84) %	Total concentration: 5.5 (2.7–11.3)[b] (range <0.9–38.5) ng/ml	Total concentration: 95.5 (46.9–153.2)[b] (range <0.9–859.7) ng/ml	Analysis of 77 paired CSF-plasma total concentrations taken at a mean of 11 h post-dosing	[38]
Zidovudine				
200 mg 8-hourly				
Total concentration CSF/plasma ratio: median range 15–120 % (interpreted from a graph)	Total concentration: range 0.12–0.17 μmol/l	Total concentration: median range 40–340 ng/ml (interpreted from a graph)	Analysis of 11 paired CSF–plasma total concentrations taken at 2–8 h post-dosing from 11 patients	[86]
Total concentration CSF/plasma ratio: 78 (6–320) %[a]	Total concentration: 93 (23–170) ng/ml[a]	Total concentration: 118 (13–740) ng/ml[a] Total concentration 3-amino-3-deoxythymidine: 2.5 (0.77–6.6) ng/ml[a]	Analysis of 23 paired CSF–plasma total concentrations taken at 2–8 h post-dosing from 23 patients	[88]
Total concentration 3-amino-3-deoxythymidine: 10 (18–23) %[a]	Total 3-amino-3-deoxythymidine: 1.7 (0.75–4.8) ng/ml[a]			
Single dose of 2.5 mg/kg intravenously				
Total concentration CSF/plasma ratio: 75 ± 26 %[c]	Total AUC_{∞}: 358 ± 200 μmol·min/l	Total AUC_6: 448 ± 213 μmol·min/l	Analysis of six paired CSF–plasma total concentrations taken up to 6 h post-dosing from six patients	[34]

AUC area under the concentration–time curve, AUC_{oral} AUC for the oral dose, AUC_6 AUC from time zero to 6 h, AUC_{12} AUC from time zero to 12 h, AUC_{∞} AUC from time zero to infinity, *CI* confidence interval, *CSF* cerebrospinal fluid, *POPPK* population pharmacokinetic

[a] Median (range)

[b] Median (interquartile range)

[c] Mean (standard deviation)

[d] Geometric means (95 % CI)

[e] Mean (range)

Table 2 Nucleoside and nucleotide reverse transcriptase inhibitors' central nervous system pharmacodynamic data

Drug	In vitro efficacy in CSF	Efficacy data	References
Abacavir	IC_{50} 0.07 μg/ml	POPPK model predicted that CSF troughs would exceed the IC_{50} for 85 % of the dose interval	[83]
		CSF C_{max} exceeded the IC_{50} by 8–20 times	[36, 37]
Lamivudine	IC_{50} (not specified)	Total CSF concentrations exceed the IC_{50}	[86]
Stavudine	IC_{50} 0.009–4.1 μmol/l	Total CSF concentrations exceed the IC_{50}	[86]
	IC_{50} 52 ng/ml	Total CSF concentrations exceed the IC_{50}	[87]
Tenofovir	IC_{50} 11.5 ng/ml	Total CSF concentrations did not exceed the IC_{50} in 77 % (59/77) of patients	[38]
Zidovudine	IC_{50} 0.002–2.400 μmol/l	Total CSF concentrations exceed the IC_{50}	[86]
	IC_{50} (not specified)	Total CSF trough concentrations exceed the IC_{50} by twofold	[34]

C_{max} maximum concentration, *CSF* cerebrospinal fluid, *IC_{50}* 50 % inhibitory concentration, *POPPK* population pharmacokinetic

limiting efficacy conclusions from total or unbound NRTI concentrations [33]. Zidovudine penetrates the CNS well, with total intravenous CNS exposure of 75 % of that in plasma [34, 35]. Approximately 35 % of total abacavir plasma concentrations penetrate the CSF [36, 37]. Lamivudine, stavudine and tenofovir CSF AUCs have not been described (see Table 1). Only 5 % of tenofovir penetrates the CSF, most likely via active transport, therefore CSF concentrations are well below the in vitro IC_{50} to suppress viral replication for most patients [38].

2.2 Non-Nucleoside Reverse Transcriptase Inhibitors

Efavirenz is more than 99.5 % protein bound, with low total efavirenz cerebrospinal exposure of less than 1 % of that of plasma; however, unbound efavirenz concentrations reach equilibrium between the two compartments (see Table 3) [14, 39]. The equilibrium between unbound concentrations in CSF and plasma is in contrast to the PIs and suggests that unbound efavirenz easily penetrates the CNS and is not actively cleared from the CNS. Efavirenz is predominantly metabolized by cytochrome P450 (CYP) 2B6 into several metabolites, of which 8-hydroxy efavirenz is the main metabolite [40, 41]. Other metabolites include 7-hydroxy efavirenz and 8,14 hydroxy efavirenz [40]. Efavirenz metabolites do not seem to inhibit viral replication but may play a role in its adverse event profile, which predominantly involves the CNS [5, 14, 40, 42]. 8-hydroxy efavirenz has been hypothesized to be implicated in neurotoxicity [5]. Extensive metabolisers may generate more 8-hydroxy efavirenz and be predisposed to develop more neurotoxicity [43]. CSF 8-hydroxy efavirenz has in fact been associated with an increase in patient neurocognitive symptoms [44]; however, no association was found between 8-hydroxy efavirenz and CYP2B6 genotype or efavirenz plasma concentration in a small study of patients of mostly Asian origin [44]. The investigators postulated that 8-hydroxy efavirenz gets trapped in the CNS. Plasma 8-hydroxy efavirenz or CNS-metabolised 8-hydroxy efavirenz may undergo glucoronidation and be unable to cross the blood–brain barrier [44]. Total and unbound efavirenz exposure in the CSF is significantly higher than the IC_{50} required to suppress viral replication (see Table 4) [14, 39, 40, 45, 46]. Efavirenz has the highest IQ_{95} of the NNRTIs [26].

Limited CSF penetration data exist for nevirapine but its drug properties may allow for good CSF penetration [47–49]. Nevirapine is the least protein bound NNRTI (60 % protein binding) and has a low molecular weight of 266.6 g/mol. The effect of CSF penetration on viral suppression has not been studied.

Etravirine is extensively protein bound (96–99.9 %) in CSF and in plasma [50]. Total etravirine concentrations in the CSF are 1–4 % of total plasma etravirine concentrations, but less than 2 % of CSF total etravirine concentration is unbound [50, 51]. The unbound etravirine concentration is well below the in vitro IC_{50} to suppress viral replication but does not seem to affect its in vivo CSF viral activity (see Table 4) [50, 51]. Nguyen et al. [50] postulated that adequate intracellular etravirine rather than unbound extracellular etravirine is required for viral suppression.

2.3 Protease Inhibitors

The PIs have a molecular weight above 500 Da and are more than 90 % plasma protein bound, with the exception of indinavir, which is less than 60 % protein bound in plasma [13, 52]. The low protein binding of indinavir translates into higher total drug concentrations in the CSF than with other PIs. Only 6 % of indinavir in the CSF is bound to proteins [13, 52]. Unbound PI concentrations in the CSF do not reach equilibrium, even at steady-state [13, 52, 53]. The lack of equilibrium is likely explained by

Table 3 Non-nucleoside reverse transcriptase inhibitors' central nervous system pharmacokinetic data

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Efavirenz				
Total concentration plasma/CSF ratio: 134 (116–198) %	Total concentration: 18.8 (9.34–22.74) ng/ml[b]	Total concentration: 2170 (1684–3953) ng/ml[b]	Analysis of 13 mid-dose paired CSF–plasma unbound and total concentrations	[14]
Unbound concentration plasma/CSF ratio: 120 (97–212) % (plasma/CSF ratio calculated, not CSF/plasma ratio)	Unbound concentration: 4.1 (2.2–4.8) ng/ml[b]	Unbound concentration: 4.8 (3.7–6.7) ng/ml[b]		
Total concentration CSF/plasma ratio: 0.5 (0.26–0.76)[b] or 0.5 %	Total concentration: 13.9 (4.1–21.2) ng/ml[b]	Total concentration: 2145 (1384–4423) ng/ml[b]	POPPK estimates of 80 mid-dose paired CSF–plasma total concentrations	[45]
POPPK estimate total concentration CSF penetration: 0.48 (0.47–0.49) % with AUC_{CSF} less than 1 % of AUC_{plasma}				
Total concentration efavirenz CSF/plasma ratio: 0.88 %	Total concentration efavirenz: 19 (7–24) ng/ml[b]	Total concentration efavirenz: 2170 (1896–2520) ng/ml[b]	Analysis of 13 mid-dose paired CSF–plasma unbound and total concentrations	[5, 40]
Total concentration 8-hydroxy efavirenz CSF/plasma ratio: 1.07 % (not reported, but calculated by authors)	Total concentration 8-hydroxy efavirenz: 3.37 (2.58–6.54) ng/ml[b] Total concentration 7-hydroxy efavirenz: undetectable Total concentration 8,14-hydroxy efavirenz: detectable in $n = 2/13$: 0.375 ng/ml and 0.444 ng/ml Unbound concentration 8-, 7- and 8,14-hydroxy efavirenz: undetectable	Total concentration 8-hydroxy efavirenz: 314.5 (206–362.3) ng/ml[b] Total concentration 7-hydroxy efavirenz: 8.84 (6.21–12.48) ng/ml[b] Total concentration 8,14-hydroxy efavirenz: 5.63 (4.58–6.16) ng/ml[b] Unbound concentration 8-, 7- and 8,14-hydroxy efavirenz: Undetectable		
Total concentration CSF/plasma AUC_{24} ratio: 0.44 (0.03–0.9) %[a] Unbound concentration CSF/plasma ratio (estimated): 88 %	Total AUC_{24}: 0.38 mg·h/l Total concentration efavirenz: 16.3 (7.3–22.3) ng/ml[a] Unbound concentration (estimated): 16.3 ng/ml	Total AUC_{24}: 86.28 mg·h/l Total concentration efavirenz: 3718 (2439–4952) ng/ml[b] Unbound concentration (estimated): 18.6 ng/ml	POPPK estimates of paired CSF–plasma total concentrations over 24 h dosing interval in one patient	[39]
Total concentration efavirenz CSF/plasma ratio: 1.07 %	Total concentration efavirenz: 10 (7.0–14.0) ng/ml[a]	Total concentration efavirenz: 936 (382–1116) ng/ml[a]	Analysis of 18 mid-dose paired CSF–plasma total concentrations	[46]
Nevirapine				
Total concentration CSF/plasma ratio: *ABCB1*-3435 C/T or T/T genotype: 62 % *ABCB*1-3435 C/C genotype: 43 %	Reported per CYP2B6 genotype	Reported per CYP2B6 genotype	Analysis of 14 paired CSF–plasma total concentrations taken around t_{max} in 11 paediatric patients	[48]
Total concentration CSF/plasma ratio: 62.6 (41–77) %[a]	Not reported	Not reported	Analysis of 16 paired CSF-plasma total concentrations.	[49]
Not calculated	Total concentration: 932 (219–1837) ng/ml[a]	Total AUC_{24}: 109,120 (52,284–190,324) ng·h/ml	Analysis of plasma and CSF total concentrations in 15 patients over a 2-year period	[47]

Table 3 continued

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Etravirine				
Total concentration CSF/plasma ratio: 1 (0.5–3) %	Total concentration: 7.24 (3.59–17.9) ng/ml[a]	Total concentration: 611.5 (148–991) ng/ml[a]	Analysis of 12 trough paired CSF–plasma total concentrations	[51]
Total concentration CSF/ unbound plasma (estimated): 1206 %		Estimated unbound concentration: 0.1 % of total (not shown)		
Total concentration CSF/plasma ratio: 4.3 (1.1–14.1)[a]; (3–5.9)[b] %	Total concentration: 9.5 (2.0–38.9)[a]; (6.4–26.4) ng/ml[b]	Total concentration: 215.2 (64–869.5)[a]; (154.1–70.4) ng/ml[b]	Analysis of 17 mid-dose paired CSF–plasma unbound and total concentrations taken over the dosing interval	[50]
Total concentration CSF/unbound plasma ratio: 101 (18–710)[a]; (76–160)[b] %	Unbound concentration: 0.13 (0.03–0.76)[a]; (0.08–0.27) ng/ml[b]	Unbound concentration: 6.2 (1.3–47.8)[a]; (4.9–34.7) ng/ml[b]		

AUC area under the concentration–time curve, AUC_{CSF} AUC of efavirenz cerebrospinal fluid concentrations, AUC_{plasma} AUC of efavirenz plasma concentrations, AUC_{24} AUC from time zero to 24 h, *CSF* cerebrospinal fluid, *CYP* cytochrome P450, *POPPK* population pharmacokinetic, t_{max} time to maximum concentration,

[a] Median (range)

[b] Median (interquartile range)

active removal of the PIs from the CSF by efflux pumps such as p-glycoprotein [13, 52].

Indinavir CSF exposure has been very well characterized, although it is no longer routinely used [54]. Table 5 summarizes the pharmacokinetic data of indinavir exposure at different dosing regimens. In vitro data suggest that unboosted indinavir reaches sufficient concentrations to inhibit wild-type virus in the majority of patients (see Table 6) [13, 55–57]. Ritonavir boosting to increase CSF concentrations specifically has been studied using indinavir [52]. Ritonavir increased plasma but not CSF unbound indinavir exposure [52]. Ritonavir has a minimal effect on p-glycoprotein at the blood–brain barrier level as low unbound concentrations reach the CNS in comparison to the gut and liver [52, 58]

Atazanavir, which has 86 % protein binding, is the PI that has the second highest proportion of unbound drug in the CSF [59]. Ritonavir added to atazanavir increases plasma total atazanavir concentrations by more than double, while CSF concentrations only increase slightly (see Table 5) [59]. The modelled estimate of total atazanavir penetration boosted with ritonavir in the CSF is 0.74 % of plasma concentrations [59]. CSF atazanavir failed to achieve concentrations above the in vitro IC_{50} in many patients (see Table 6). Unboosted atazanavir has the lowest IQ_{95} of the PIs [26]. Additional ritonavir increases the IQ_{95} of atazanavir similar to that of boosted lopinavir [26].

Nelfinavir manufacturing has been discontinued and no longer available as a treatment option. It is highly protein bound (99.7 ± 0.10 %) and reaches undetectable CSF concentrations, mostly when measured [57, 60–63]. Total nelfinavir CSF exposure in relation to plasma has not been adequately quantified despite sensitive methodology and instrumentation (see Table 5) [63, 64]. CSF nelfinavir concentrations are in the range of in vitro inhibitory concentrations of wild-type virus (see Table 6) [63, 64].

When boosted with ritonavir, lopinavir reaches therapeutic concentrations in plasma. Lopinavir is 97–99 % protein bound, with less than 0.5 % of total lopinavir concentrations reaching the CSF (see Table 5) [46, 60, 61, 65, 66]. Lopinavir total CSF concentrations exceed in vitro concentrations required to inhibit wild-type virus [46, 65, 66]. Data on lopinavir AUC exposure and CSF unbound concentrations are lacking.

Darunavir is only 6.5 % unbound in plasma and 97.2 % in CSF [53]. At the darunavir/ritonavir dose of 600/100 mg, total darunavir CSF concentrations are approximately 1 % of total plasma concentrations (see Table 5) [53, 67, 68]. Unbound darunavir CSF concentrations are significantly higher at 8.5 % of unbound plasma concentrations [53]. Darunavir has adequate CSF exposure (see Table 6) and the highest IQ_{95} of all the evaluated antiretrovirals [26].

Table 4 Non-nucleoside reverse transcriptase inhibitors' central nervous system pharmacodynamic data

Drug	In vitro efficacy in CSF	Efficacy data	References
Efavirenz	IC_{50} 0.51 ng/ml	CSF unbound above the wild-type in vitro IC_{50} in lymphocytes of 0.51 ng/ml, and CSF total concentrations exceeded the wild-type IC_{50} in lymphocytes of 0.51 ng/ml by a ratio of 26 (8–41)[a]	[14, 45]
		CSF total concentrations exceeded the IC_{50} in 14/18 (78 %) patients	[46]
	IC_{50} 0.36 ng/ml	CSF total concentration above the IC_{50}. Metabolites (8-, 7- and 8,14-OH) considered to be minimally effective at inhibiting viral replication. CSF concentrations below the IC_{50} for 8-OH efavirenz (42.25 ng/ml), 7-OH efavirenz (44.68 ng/ml) and 8,14-OH efavirenz (2238.4 ng/ml)	[40]
	IC_{50} 1.3 ng/ml	CSF total concentration exceeded the wild-type IC_{50} in lymphocytes in a protein-free medium of 1.3 ng/ml by 12-fold	[39]
Etravirine	IC_{50} range of 0.39–2.4 ng/ml	CSF total concentrations exceeded the IC_{50} range	[51]
	IC_{50} of 0.9 ng/ml	CSF total concentrations exceeded the vitro unbound IC_{50} for wild-type HIV-1 of 0.9 ng/ml, but unbound CSF concentrations were all below the IC_{50}	[50]

CSF cerebrospinal fluid, *IC_{50}* 50 % inhibitory concentration

[a] Interquartile range

The unbound plasma fraction of saquinavir is less than 1 % [69, 70]. CSF unbound concentrations are mostly unmeasurable, and when measured the unbound saquinavir CSF:plasma ratio is less than 1 % [69]. CSF concentrations are below the in vitro concentrations required to inhibit wild-type virus (see Table 6) [63, 69, 71].

2.4 Other Antiretroviral Drugs

The CSF concentrations of the fusion inhibitor enfuvirtide are not quantifiable due to negligible CSF penetration [72]. Although no unbound AUC penetration data are available, total CSF and plasma paired samples indicate that the entry inhibitor maraviroc and the integrase inhibitor raltegravir enter the CSF. Maraviroc achieves total CSF concentrations in excess of threefold the effective concentration to inhibit viral replication of 0.57 ng/ml [73]. In seven paired total CSF and plasma concentrations the median and range of plasma and CSF concentrations were 94.9 (21.4–478) and 3.63 (1.83–12.2) ng/ml, respectively, giving a median CSF/plasma ratio of 3 % (1–10) [73]. Raltegravir total CSF concentrations are approximately 6.0 % that of plasma, and exceed the concentration required to inhibit 50 % of viral replication in all patients but fail to exceed the IC_{95} in at least half of the patients (see Tables 7, 8) [74, 75].

3 Pharmacogenetic Data

A spectrum of transporters, classified into ATP-binding cassette (ABC) or solute-carrier (SLC) transporters, exist to facilitate or prevent the movement of molecules across the blood–CNS interface. Transport of ART out of the CNS is mediated by p-glycoprotein (also known as MDR-1 or ABCB1), the multidrug resistance-associated proteins (or MRPs, also known as ABCC) and breast cancer resistance protein (BCRP, also known as ABCG2) [17, 76, 77]. Limited data are available on the SLC superfamily at the blood–brain barrier, but they also seem to play an important role in the efflux of molecules [78]. Although CYP1B1 has been detected at the human blood–brain barrier, CYP3A4, CYP2C9 and CYP2D6 have not, and the impact of the enzymatic barrier on cerebral disposition of ART is probably negligible [18]. Genetic polymorphisms in ART blood–brain barrier transporters may therefore contribute to the difference in CNS ART exposure [19, 79]. Patient data to support the contribution of blood–brain barrier transporter polymorphisms to CNS antiretroviral concentrations are currently limited (see Table 9) and plagued by the lack of power to detect true associations [80].

4 Discussion and Conclusion

We reviewed the CNS pharmacokinetic, pharmacodynamic and pharmacogenetic data of ART. The movement of drug molecules into the CNS is complex, and extrapolation of CNS drug exposure from CSF drug concentrations oversimplifies the pharmacokinetics of CNS ART; however, CSF is the most accessible CNS matrix [10]. The majority of published ART CNS penetration studies measured CNS penetration by using single paired CSF–plasma

Table 5 Protease inhibitors' central nervous system pharmacokinetic data

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Indinavir				
800 mg 8-hourly without ritonavir				
Not stated	Total concentration: 223 (200)[c] (range 80–660) nmol/l	Total concentration: 2086 (3400)[c] (range 70–10,300) nmol/l	Analysis of 32 paired CSF–plasma total concentrations at various time points during the dosing interval from 25 patients	[89]
Total concentration CSF/plasma AUC_8 ratio: 6.5 (1.0)[c] (range 4.9–8.0) % Unbound concentration CSF/plasma AUC_8 ratio: 14.7 (2.6)[c] (range 10.3–17.7) %	Total AUC_8: 1720 (538)[c] (range 886–2256) nmol·h/l Unbound AUC_8: 1616 (493)[c] (range 861–2172) nmol·h/l	Total AUC_8: 26,939 (8908)[c] (range 11,845–38,930) nmol·h/l Unbound AUC_8: 11,218 (3780)[c] (range 5165–16,974) nmol·h/l	Analysis of 80 paired CSF–plasma unbound and total concentrations over 8 h dosing interval from eight patients	[13]
Total concentration CSF/plasma ratio: 6 (95 % CI 5–9) %	Total concentration: 145 (43–480) nM[a]	Total concentration: 1491 (40–11,670) nM[a]	POPPK analysis of 22 paired CSF–plasma total concentrations at various time points during the dosing interval from 22 patients	[56]
Total concentration CSF/plasma ratio: 1.7 (0.2–5.1) %[d]	Total concentration: 0.11 (0.032–0.25) μmol/l[d]	Total concentration: mean 10.28 μmol/l	Analysis of 19 paired CSF–plasma total concentrations at various time points during the dosing interval from 19 patients	[90]
Total concentration CSF/plasma ratio: median 14 %	Total concentration: 86 (13)[c]; median 81 ng/ml	Total concentration: 485 (137)[c]; median 283 ng/ml	Analysis of 16 paired CSF–plasma total concentrations at various time points during the dosing interval from 16 patients	[60]
Total concentration CSF/plasma ratio: 17 (10–49) %[b]	Total concentration: 73 (52–92) ng/ml[b]	Total concentration: 357 (155–914) ng/ml[b]	Analysis of 28 paired CSF–plasma total concentrations at various time points during the dosing interval from 14 patients	[61]
800 mg 8-hourly with 100 mg ritonavir 12-hourly				
Not stated	Total concentration: 104 (68–207) ng/ml[b] with ritonavir: 100 mg ritonavir dosed with 800 mg indinavir 8- or 12-hourly	Total AUC_8: 29,035 (25,559–30,496) ng·h/ml[b] Total AUC_{24}: 87,105 (76,677–91,488) ng·h/ml[b]	Analysis of four paired CSF–plasma total concentrations taken 1 h after dosing from four patients	[55]
800 mg 12-hourly with 100 mg ritonavir 12-hourly				
Not stated	Total concentration: 104 (68–207) ng/ml[b] with ritonavir: 100 mg ritonavir dosed with 800 mg indinavir 8- or 12-hourly	Total AUC_{12}: 30,121 (24,352–38,438) ng·h/ml[b] Total AUC_{24}: 60,242 (48,704–76,876) ng·h/ml[b]	Analysis of four paired CSF–plasma total concentrations taken 1 h after dosing from four patients	[55]
Total concentration CSF/plasma ratio: 9.9 (3.3)[c] (range 7.4–16.6) % Unbound concentration CSF/plasma ratio: 17.5 (6.4)[c] (range 12.8–31.4) %	Total AUC_{12}: 6606 (2481)[c] (range 3903–11,385) nmol·h/l Unbound AUC_{12}: 6502 (2397)[c] (range 3903–11,043) nmol·h/l	Total AUC_{12}: 68,913 (23,302)[c] (range 50,404–117,049) nmol·h/l Unbound AUC_{12}: 38,829 (15,124)[c] (range (26,614–71,283) nmol·h/l	Analysis of 63 paired CSF–plasma unbound and total concentrations over 8 h dosing interval taken from seven patients	[52]
1000 mg 8-hourly without ritonavir				

Table 5 continued

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Not stated	Total concentration: 39 (27–54) ng/ml[b] without ritonavir Total concentration: 104 (68–207) ng/ml[b] with ritonavir: 100 mg ritonavir dosed with 800 mg indinavir 8- or 12-hourly	Total AUC_8: 16,474 (13,624–19,481) ng·h/ml[b] Total AUC_{24}: 49,422 (40,873–58,442) ng·h/ml[b]	Analysis of 11 paired CSF–plasma total concentrations taken 1 h after dosing taken from 11 patients	[55]
Not stated	Total concentration: median 71 ng/ml	Not stated	Analysis of 17 paired CSF–plasma total concentrations at various time points during the dosing interval from 11 patients	[57]
Atazanavir				
400 mg daily				
Total concentration CSF/plasma ratio: 1.12 (0.5–13.9) %[a]	Total concentration: 7.9 (<5 to 40) ng/ml[a]	Total concentration: 523 (<128 to 6200) ng/ml[a]	Analysis of nine paired CSF–plasma total concentrations at various time points during the dosing interval	[59]
300 mg daily with ritonavir				
Total concentration CSF/plasma ratio: 0.74 %	Total concentration: 10.3 (<5 to 38) ng/ml[a]	Total concentration: 1278 (<128 to 5295) ng/ml[a]	POPPK analysis of 62 paired CSF–plasma total concentrations at various time points during the dosing interval	[59]
400 mg daily and 300 mg daily with ritonavir				
Not stated	Total concentration: 14.5 (1.9–17.5) ng/ml[b] Extrapolated C_{min} 7.3 (1.9–10.4) ng/ml[b]	Total concentration: 700 (470–964) ng/ml[b] Extrapolated C_{min} 265 (177–447) ng/ml[b]	Analysis of 12 paired CSF–plasma total concentrations at a median post-dose sampling interval of 15.5 h	[46]
Total concentration CSF/plasma ratio: 0.9 (0.8)[c] (range 0.1–2.7) %	Total concentration: 8.3 (0.6–40) ng/ml[a]	Total concentration: 1250 (205–3555) ng/ml[a]	Analysis of 22 paired CSF–plasma total concentrations at various time points during the dosing interval from 22 patients	[91]
Nelfinavir				
Not stated	Total concentration: <2.0 (<2.0 to 23.0) nM[a] (detectable in 9 of 15 samples)	Total concentration: 4.1 (<0.13 to 10.6) μM[a] Unbound concentration: 10.0 (<2.0 to 31.0) nM[a]	Analysis of 15 paired CSF–plasma unbound and total concentrations at different intervals from eight patients	[63]
Not stated	Total concentration: 9 (6–29) nM[a] (detectable in 16 of 18 samples and quantifiable in 8 of 18 samples)	Not stated	Analysis of 18 paired CSF–plasma unbound and total concentrations at various time points during the dosing interval from 18 patients	[64]
Lopinavir				
Total concentration CSF/plasma ratio: 0.225 (0.194–0.324) %[b]	Total concentration: 11,200 (6760–16,400) pg/ml[b]	Total AUC_{12}: 71.3 (48.4–87.6) μg·h/ml[b]	Analysis of ten paired CSF–plasma total trough concentrations from ten patients	[66]

Table 5 continued

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Total concentration CSF/plasma ratio: 0.23 (0.12–0.75) %[b]	Total concentration: 17.0 (12.1–22.7) μg/l[b]	Total concentration: 5889 (4805–9620) μg/l[b]	Analysis of 31 paired CSF–plasma total concentrations at a median post-dose sampling interval of 4.3 h from 26 patients	[65]
Not calculable	Undetectable	Total concentration: 5463 (720) ng/ml[c]	Analysis of 16 paired CSF–plasma total trough concentrations from 16 patients	[60]
Not stated	Total concentration: 23 (17.5–40)[b] ng/ml Extrapolated C_{min}18.4 (10.5–29.3)[b] ng/ml	Total concentration: 5435 (4049–7816) ng/ml[b] Extrapolated C_{min} 3566 (2579–5388) ng/ml[b]	Analysis of 42 paired CSF–plasma total concentrations at a median post-dose sampling interval of 6 h	[46]
Not calculable	Undetectable	Total concentration C_{min}: 5863 (3505–7453) ng/ml[b]	Analysis of 12 paired CSF–plasma total trough concentrations from 12 patients	[61]
Darunavir				
800 mg and 100 mg ritonavir daily				
Total concentration CSF/plasma ratio: 0.32 (0.25–0.44) %[b]	Total concentration: 10.7 (6.7–23) ng/ml[b]	Not separately reported	Analysis of nine paired CSF–plasma total trough concentrations	[68]
600 mg and 100 mg ritonavir 12-hourly				
Total concentration CSF/plasma ratio: 0.9 (0.60–1.53) %[b]	Total concentration: 38.2 (30.2–52.3) ng/ml[b]	Not separately reported	Analysis of 14 paired CSF–plasma total trough concentrations	[68]
Total concentration CSF/plasma ratio: 1.4 (0.9–1.8)[b] (range 0.3–2.6) % Unbound concentration CSF/plasma ratio: 8.5 (6.2–12.7)[b] (range 2.9–412.4) %	Total concentration: 55.8 (39.5–79.1)[b] (range 19.4–159.6) ng/ml Unbound concentration: 50.2 (35.0–72.6)[b] (range 0–143.8) ng/ml	Total concentration: 4094 (2993–6411)[b] (range 104–11,298) ng/ml Unbound concentration: 538 (369–968)[b] (range 1–2206) ng/ml	Analysis of 29 paired CSF–plasma unbound and total concentrations at various time points during the dosing interval from 16 patients	[53]
Total concentration CSF/plasma ratio: 0.9 (0.3–1.8) %[b]	Total concentration: 34.2 (15.9–212) ng/ml[b]	Total concentration: 3930 (1800–12,900) ng/ml[a]	Analysis of 14 paired CSF–plasma total concentrations at various time points during the dosing interval from eight patients	[67]
Saquinavir				
Dosing regimen not stated				
Total concentration CSF/plasma ratio: 0.1 and 0.2 %, respectively	Total concentration: measured in 2/11 participants: 0.3 and 1.6 ng/ml, respectively	Not stated	Analysis of 11 paired CSF-plasma total concentrations at 6–8 h post-dosing from 11 patients	[71]
Not stated	Total concentration: <2.5 (<2.5 to 9.0) nM[a] (detectable in 7 of 15 samples)	Total concentration: 300 (<80 to 6600) nM[a] Unbound concentration: <2.5 (<2.5 to 96.0) nM[a]	Analysis of 15 paired CSF–plasma unbound and total concentrations at different intervals from eight patients	[63]
400 mg with 400 mg ritonavir 12-hourly				
Unbound concentration CSF/plasma ratio: 0.16 ± 0.09 %[c]	Unbound concentration: 0.40 ± 0.30 ng/ml[c] (detectable in 5 of 12 samples)	Unbound concentration: 6.8 ± 9.5 ng/ml[c]	Analysis of 12 mid-dose paired CSF–plasma unbound and total concentrations	[69]

Table 5 continued

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
600 mg with food 8-hourly				
Not calculable	Undetectable	Total concentration: 167 ng/ml	Analysis of five mid-dose paired CSF–plasma total concentrations	[70]
600 mg with ritonavir 12-hourly				
0.3 %	6.5 ng/ml	Total concentration: 1094 ng/ml	Analysis of four mid-dose paired CSF–plasma total concentrations	[70]
Calculated in $n = 1$	Detectable in $n = 1$			

AUC area under the concentration–time curve, *AUC8* AUC from time zero to 8 h, *AUC12* AUC from time zero to 12 h, *AUC24* AUC from time zero to 24 h, C_{min} minimum concentration during dosing interval, *CI* confidence interval, *CSF* cerebrospinal fluid, *POPPK* population pharmacokinetic

[a] Median (range)

[b] Median (interquartile range)

[c] Mean (standard deviation)

[d] Mean (range)

concentration points to determine exposure. AUC accurately estimates exposure, which can only be determined by using multiple paired CSF–plasma concentrations in a patient or by combining samples from multiple patients using a population pharmacokinetic approach. Indinavir and efavirenz CNS penetration was characterized by measuring unbound plasma and CSF concentrations to calculate AUCs. The importance of measuring total and unbound drug concentrations is illustrated by efavirenz, where the CSF total concentration is a tiny fraction of plasma total concentration, while unbound concentrations in the two compartments are similar [14]. The unbound concentration of indinavir in the CSF is double that of the total concentration in the CSF [81]. Accurate inferences of ART CNS penetration without unbound AUC data in plasma and CSF compartments are limited.

Future studies should measure total and unbound antiretroviral concentrations and aim to calculate AUCs as a measure of exposure. High CPE score ART CNS exposure is a risk factor for HIV-D, suggesting that a therapeutic window does indeed exist for ART in the CNS where concentrations at the higher and lower spectrum lead to ART toxicity or viral replication, respectively [7, 27]. Laboratory evidence suggests that antiretrovirals are directly neurotoxic [5, 6]. Neurons challenged for a week with different concentrations of antiretrovirals, including therapeutic concentrations, underwent structural loss, as quantified using microtubule-associated protein-2 [6]. Neurotoxicity was most pronounced with abacavir, atazanavir, efavirenz, etravirine and nevirapine. Of particular interest is the inactive metabolite of efavirenz (8-hydroxy efavirenz), which was tenfold more toxic than efavirenz in rat neuronal cultures and has been associated with more CNS symptoms in patients [5, 44]. Future studies should quantify ART CNS therapeutic ranges not only to determine which antiretroviral drugs penetrate the CNS adequately to suppress viral replication but also which antiretroviral drugs penetrate the CNS to such an extent that they contribute to neurotoxicity. The impact of genetic polymorphisms in drug transport across membranes (including the blood–brain barrier) is well established for many drugs, including ART [21, 22, 82]. However, data on the clinical relevance and extent of the contribution of polymorphisms in genes encoding for blood–brain transporters to CNS antiretroviral exposure are limited due to the small number of studies and the lack of power. The invasiveness of lumbar punctures limits the sample size of CSF exposure studies. Correlations between CNS antiretrovira lexposure and effect is multifaceted. To accurately predict CNS effects there is a need to develop a sophisticated intra-brain pharmacokinetic–pharmacodynamic–pharmacogenetic model that includes transporters as well as the influence of HIV.

Table 6 Protease inhibitors' central nervous system pharmacodynamic data

Drug	In vitro efficacy in CSF	Efficacy data	References
Indinavir	IC_{95} 30–60 ng/ml MEC 40 ng/ml	Total CSF concentrations exceeded IC_{95} and MEC when indinavir is dosed with ritonavir. Without ritonavir, patients may have CSF total concentrations below IC_{95} and MEC	[55]
	IC_{95} 25–100 nmol/l	Unbound CSF concentrations exceeded the IC_{95} for 85 % of the dosing interval in seven of eight participants with a C_{min} 122 (51)[a] (range 49–204 nmol/l)	[13]
		Median total CSF concentration exceeded the IC_{95}	[89]
		Unbound CSF concentrations exceeded the IC_{95} for 100 % of the dosing interval in all participants with a C_{min} 280 (131)[a] (range 149–527 nmol/l)	[52]
		All total CSF concentrations exceeded 25 nmol/l, while only 54 % (12/22) of patients exceeded 100 nM	[56]
	IC_{95} 18–70 ng/ml	Median total CSF concentration exceeded IC_{95}	[57]
Atazanavir	IC_{50} 1 ng/ml	Total CSF concentrations were near the IC_{50} in 16 % (11/67) of samples	[59]
		Total CSF concentrations were below the IC_{50} in 17 % (2/12) of samples	[46]
		Total CSF concentrations were considered to be above the IC_{50} in general	[91]
Nelfinavir	IC_{50} (not specified)	Detectable CSF concentrations were in the range of IC_{50} for wild-type virus	[63]
	IC_{95} 0.35–10 nM	Unbound nelfinavir in CSF in the concentration range of the IC_{95} in some of the CSF samples (8/18)	[64]
Lopinavir	IC_{50} 1.9 μg/l (3.0 nmol/)	Total CSF concentrations exceeded IC_{50} for wild-type replication by a median (IQR) 5.9-fold (3.9–8.6)	[66]
		Total CSF concentrations exceeded IC_{50} for wild-type replication by a median (IQR) 5.3-fold (3.8–7.2)	[65]
		Extrapolated trough concentrations above IC_{50} for 98 % (41/42) of CSF samples	[46]
	CSF HIV-1 RNA levels	HIV-1 RNA levels detectable in 3 of 10 patients (median 350 copies/ml)	[60]
		HIV-1 RNA levels detectable in 3 of 12 patients (median 350 copies/ml)	[61]
Darunavir	IC_{50} 2.75 ng/ml	None of the patients receiving darunavir/ritonavir 600/100 mg 12-hourly compared with 12 % receiving 800/100 mg daily were below the IC_{50}	[68]
	IC_{50} 1.78 ng/ml IC_{90} 2.43 ng/ml	All unbound CSF concentrations exceeded the IC_{50} and IC_{90} wild-type HIV-1 by a median of 28.1-fold and 20.6-fold, respectively	[53]
	IC_{50} 12–55 ng/ml	Total CSF concentrations were in the range of, or exceeded, the IC_{50}	[67]
Saquinavir	IC_{50} 42–55 ng/ml	Detectable CSF concentrations were below the IC_{50} for wild-type virus	[63, 69, 71]

C_{min} minimum concentration during dosing interval, *CSF* cerebrospinal fluid, *IC_{95}* 95 % inhibitory concentration, *IC_{50}* 50 % inhibitory concentration, *IC_{90}* 90 % inhibitory concentration, *IQR* interquartile range, *MEC* minimal effective concentration,

[a] Mean (standard deviation)

Table 7 Integrase inhibitors' central nervous system pharmacokinetic data

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
Raltegravir				
400 mg 12-hourly				
CSF/plasma total concentration ratio: 3 (1–61) %[a]	Total concentration: 18.4 (<2.0 to 126) ng/ml[a]	Total concentration: 448 (37–5180) ng/ml[a]	Analysis of 25 paired CSF–plasma total concentrations taken at 1.2–14 h post-dosing from 16 patients	[74]
CSF/plasma total concentration ratio: 5.8 (2.1–17.8)[c] (range 1–53.5) %	Total concentration: 14.5 (9.3–26.1)[c] (range 6.0–94.2) ng/ml	Total concentration: 260.9 (72–640.4)[c] (range 17.8–4870) ng/ml	Analysis of 22 paired CSF–plasma total concentrations taken at 6.1 ± 4.2 h[b] post-dosing from 18 patients	[92]
Plasma AUC_{12}/CSF_{4h} total concentration ratio: 6.0 ± 2.6 %[b]	Total concentration: 30.1 (17.0 50.4) ng/ml[c]	Total AUC_{12}: 6.55 (3.48–13.0) h·mg/ml[c]	Analysis of 40 paired CSF–plasma total concentrations taken at 4 h post-dosing from 40 participants	[80]

Table 7 continued

Reported CSF penetration in relation to plasma exposure	CSF exposure	Plasma exposure	Methodology sample (CSF–plasma)	References
CSF/plasma total concentration ratio: 20.6 (3.8–36.3)[c] (range 0.5–133) %	Total concentration: 31.0 (21–56) ng/ml[c]	Total concentration: 165 (83–552) ng/ml[c]	Analysis of 41 paired CSF–plasma total concentrations taken at 2–15 h post-dosing from 41 participants	[25]

AUC area under the concentration–time curve, AUC_{12} AUC from time zero to 12 h, *CSF* cerebrospinal fluid, CSF_{4h} cerebrospinal fluid concentration at 4 hours

[a] Median (range)

[b] Mean (standard deviation)

[c] Median (interquartile range)

Table 8 Integrase inhibitors' central nervous system pharmacodynamic data

Drug	In vitro efficacy in CSF	Efficacy data	References
Raltegravir	IC_{95} 9–15 ng/ml	Total CSF concentrations exceed the IC_{95} in half of the patients (13/25)	[74]
	IC_{50} 3.2 ng/ml IC_{95} 9–15 ng/ml	Total CSF concentrations exceed the IC_{50} by a median of 4.5-fold, and little less than half of the patients (10/21) exceeded the IC_{95}	[92]
		Total CSF concentrations exceeded the IC_{50} in all patients, and 28.6 % (10/35) exceeded the IC_{95}	[25]

CSF cerebrospinal fluid, IC_{50} 50 % inhibitory concentration, IC_{95} 95 % inhibitory concentration,

Table 9 Pharmacogenetic associations with central nervous system antiretroviral exposure

Drug	Genetic associations explored	Findings	References
Nevirapine	*ABCB1* 3435 C>T *CYP2B6*-G516T	ABCB1-C3435T C/T or T/T genotypes ($n = 9$) associated with higher nevirapine CSF/plasma ratios compared with ABCB1-C3435T C/C genotype ($n = 5$); $p = 0.01$. No significant difference was observed when the ratios were compared with the CYP2B6-G516T genotype ($p = 1.00$)	[48]
Darunavir	*ABCB1* 3435 C>T *ABCB1* 1236 C>T *ABCB1* 2677 G>T *ABCC2* −24 G>A ***SLCO1A2* 38 A>G** *SLCO1A2* 516 A>C	AA compared with AG genotype-carrying patients showed a trend towards higher CSF concentrations and plasma/CSF ratios: 32 (22–72) ng/ml[a] vs. 29.8 (139–36.4) ng/ml[a] ($p = 0.13$) and 0.78 (0.78–1.88) %[a] vs. 0.56 (0.35–0.96) %[a] ($p = 0.13$), respectively	[68]
Nelfinavir	*ABCB1* 3435 C>T *ABCB1* 2677 G>A/T	CC 3435 genotype occurred more frequently in patients with undetectable CSF viral loads (6/7 compared with 6/12), but the finding was not statistically significant	[64]
Raltegravir	*ABCB1* 3435 C>T	No significant association between ABCB1 3435 C>T and the CSF/plasma AUC or concentration	[80]
	ABCB1 3435 C>T *ABCB1* 1236 C>T *ABCB1* 2677 G>A/T *SLCO1A2* 38 A>G *SLCO1A2* 516 A>C *ABCC2* 24 G>A *SLC22A6* 453 G>A $HNF4_{\alpha}$ 613 C>G	No significance between selected SNPs and CSF/plasma ratios	[25]

Bolded text denotes an association

AUC area under the concentration–time curve, *CSF* cerebrospinal fluid, *CYP* cytochrome P450, *SNPs* single nucleotide polymorphisms

[a] Median (interquartile range)

Acknowledgments Eric Decloedt, Bernd Rosenkranz, Gary Maartens and John Joska have no conflicts of interest to declare and no funding was received to prepare this manuscript. Eric Decloedt and John Joska received self-initiated research funding from the European and Developing Countries Clinical Trials Partnership (EDCTP Grant number SP.2011.41304.065) and the South African Medical Research Council.

References

1. Heaton RK, Clifford DB, Franklin DR, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. Neurology. 2010;75(23):2087–96.
2. Sacktor N, Robertson K. Evolving clinical phenotypes in HIV-associated neurocognitive disorders. Curr Opin HIV AIDS. 2014;9(6):517–20.
3. Andrade AS, Deutsch R, A Celano S, et al. Relationships among neurocognitive status, medication adherence measured by pharmacy refill records, and virologic suppression in HIV-infected persons. J Acquir Immune Defic Syndr. 2013;62(3):282–92.
4. Letendre S. Central nervous system complications in HIV disease: HIV-associated neurocognitive disorder. Top Antivir Med. 2011;19(4):137–42.
5. Tovar-y-Romo LB, Bumpus NN, Pomerantz D, et al. Dendritic spine injury induced by the 8-hydroxy metabolite of efavirenz. J Pharmacol Exp Ther. 2012;343(3):696–703.
6. Robertson K, Liner J, Meeker RB. Antiretroviral neurotoxicity. J Neurovirol. 2012;18(5):388–99.
7. Caniglia EC, Cain LE, Justice A, et al. Antiretroviral penetration into the CNS and incidence of AIDS-defining neurologic conditions. Neurology. 2014;83(2):134–41.
8. Letendre S, Marquie-Beck J, Capparelli E, et al. Validation of the CNS penetration-effectiveness rank for quantifying antiretroviral penetration into the central nervous system. Arch Neurol. 2008;65(1):65–70.
9. Nau R, Sörgel F, Eiffert H. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clin Microbiol Rev. 2010;23(4):858–83.
10. De Lange ECM, Danhof M. Considerations in the use of cerebrospinal fluid pharmacokinetics to predict brain target concentrations in the clinical setting: implications of the barriers between blood and brain. Clin Pharmacokinet. 2002;41(10):691–703.
11. Enting RH, Hoetelmans RM, Lange JM, Burger DM, Beijnen JH, Portegies P. Antiretroviral drugs and the central nervous system. AIDS. 1998;12(15):1941–55.
12. Wynn HE, Brundage RC, Fletcher CV. Clinical implications of CNS penetration of antiretroviral drugs. CNS Drugs. 2002;16(9):595–609.
13. Haas DW, Stone J, Clough LA, et al. Steady-state pharmacokinetics of indinavir in cerebrospinal fluid and plasma among adults with human immunodeficiency virus type 1 infection. Clin Pharmacol Ther. 2000;68(4):367–74.
14. Avery LB, Sacktor N, McArthur JC, Hendrix CW. Protein-free efavirenz concentrations in cerebrospinal fluid and blood plasma are equivalent: applying the law of mass action to predict protein-free drug concentration. Antimicrob Agents Chemother. 2013;57(3):1409–14.
15. Boffito M, Back DJ, Blaschke TF, et al. Protein binding in antiretroviral therapies. AIDS Res Hum Retrovir. 2003;19(9):825–35.
16. Ronaldson PT, Persidsky Y, Bendayan R. Regulation of ABC membrane transporters in glial cells: relevance to the pharmacotherapy of brain HIV-1 infection. Glia. 2008;56(16):1711–35.
17. Varatharajan L, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary of current knowledge and recommendations for further research. Antivir Res. 2009;82(2):A99–109.
18. Eyal S, Hsiao P, Unadkat JD. Drug interactions at the blood-brain barrier: fact or fantasy? Pharmacol Ther. 2009;123(1):80–104.
19. Löscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci. 2005;6(8):591–602.
20. Groothuis DR, Levy RM. The entry of antiviral and antiretroviral drugs into the central nervous system. J Neurovirol. 1997;3:387–400.
21. Cropp CD, Yee SW, Giacomini KM. Genetic variation in drug transporters in ethnic populations. Clin Pharmacol Ther. 2008;84(3):412–6.
22. Rodríguez-Nóvoa S, Barreiro P, Jiménez-Nácher I, Soriano V. Overview of the pharmacogenetics of HIV therapy. Pharmacogenomics J. 2006;6(4):234–45.
23. Avison MJ, Nath A, Greene-Avison R, Schmitt FA, Greenberg RN, Berger JR. Neuroimaging correlates of HIV-associated BBB compromise. J Neuroimmunol. 2004;157(1–2):140–6.
24. Calcagno A, Di Perri G, Bonora S. Pharmacokinetics and pharmacodynamics of antiretrovirals in the central nervous system. Clin Pharmacokinet. 2014;53:891–906.
25. Calcagno A, Cusato J, Simiele M, et al. High interpatient variability of raltegravir CSF concentrations in HIV-positive patients: a pharmacogenetic analysis. J Antimicrob Chemother. 2014;69(1):241–5.
26. Calcagno A, Simiele M, Alberione MC, et al. Cerebrospinal fluid inhibitory quotients of antiretroviral drugs in HIV-infected patients are associated with compartmental viral control. Clin Infect Dis. 2014;60:311–7.
27. Letendre SL, Ellis RJ, Ances BM, McCutchan JA. Neurologic complications of HIV disease and their treatment. Top HIV Med. 2010;18(2):45–55.
28. Marra CM, Zhao Y, Clifford DB, et al. Impact of combination antiretroviral therapy on cerebrospinal fluid HIV RNA and neurocognitive performance. AIDS. 2009;23(11):1359–66.
29. Cysique LA, Vaida F, Letendre S, et al. Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. Neurology. 2009;73(5):342–8.
30. Tozzi V, Balestra P, Salvatori MF, et al. Changes in cognition during antiretroviral therapy: comparison of 2 different ranking systems to measure antiretroviral drug efficacy on HIV-associated neurocognitive disorders. J Acquir Immune Defic Syndr. 2009;52(1):56–63.
31. Giunta B, Ehrhart J, Obregon DF, et al. Antiretroviral medications disrupt microglial phagocytosis of β-amyloid and increase its production by neurons: implications for HIV-associated neurocognitive disorders. Mol Brain. 2011;4(1):23.
32. Ellis RJ, Letendre S, Vaida F, et al. Randomized trial of central nervous system-targeted antiretrovirals for HIV-associated neurocognitive disorder. Clin Infect Dis. 2014;58(7):1015–22.
33. Bazzoli C, Jullien V, Le Tiec C, Rey E, Mentré F, Taburet A-M. Intracellular pharmacokinetics of antiretroviral drugs in HIV-infected patients, and their correlation with drug Action. Clin Pharmacokinet. 2010;49(1):17–45.
34. Rolinski B, Bogner JR, Sadri I, Wintergerst U, Goebel FD. Absorption and elimination kinetics of zidovudine in the cerebrospinal fluid in HIV-1-infected patients. J Acquir Immune Defic Syndr Hum Retrovirol. 1997;15(3):192–7.
35. Fischl MA, Richman DD, Grieco MH, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N Engl J Med. 1987;317(4):185–91.

36. McDowell JA, Chittick GE, Ravitch JR, Polk RE, Kerkering TM, Stein DS. Pharmacokinetics of [14C]abacavir, a human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitor, administered in a single oral dose to HIV-1-infected adults: a mass balance study. Antimicrob Agents Chemother. 1999;43:2855–61.
37. McDowell JA, Lou Y, Symonds WS, Stein DS. Multiple-dose pharmacokinetics and pharmacodynamics of abacavir alone and in combination with zidovudine in human immunodeficiency virus-infected adults. Antimicrob Agents Chemother. 2000;44(8):2061–7.
38. Best BM, Letendre SL, Koopmans P, et al. Low cerebrospinal fluid concentrations of the nucleotide HIV reverse transcriptase inhibitor, tenofovir. J Acquir Immune Defic Syndr. 2012;59(4):376–81.
39. Yilmaz A, Watson V, Dickinson L, Back D. Efavirenz pharmacokinetics in cerebrospinal fluid and plasma over a 24-h dosing interval. Antimicrob Agents Chemother. 2012;56(9):4583–5.
40. Avery LB, VanAusdall JL, Hendrix CW, Bumpus NN. Compartmentalization and antiviral effect of efavirenz metabolites in blood plasma, seminal plasma, and cerebrospinal fluid. Drug Metab Dispos. 2013;41(2):422–9.
41. Di Iulio J, Fayet A, Arab-Alameddine M, et al. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. Pharmacogenet Genomics. 2009;19(4):300–9.
42. Kenedi CA, Goforth HW. A systematic review of the psychiatric side-effects of efavirenz. AIDS Behav. 2011;15(8):1803–18.
43. Decloedt EH, Maartens G. Neuronal toxicity of efavirenz: a systematic review. Expert Opin Drug Saf. 2013;12(6):841–6.
44. Winston A, Amin J, Clarke A, et al. Cerebrospinal fluid exposure of efavirenz and its major metabolites when dosed at 400 mg and 600 mg once daily: a randomized controlled trial. Clin Infect Dis. 2014. (Epub 11 Dec).
45. Best BM, Koopmans PP, Letendre SL, et al. Efavirenz concentrations in CSF exceed IC50 for wild-type HIV. J Antimicrob Chemother. 2011;66(2):354–7.
46. Cusini A, Vernazza PL, Yerly S, et al. Higher CNS penetration-effectiveness of long-term combination antiretroviral therapy is associated with better HIV-1 viral suppression in cerebrospinal fluid. J Acquir Immune Defic Syndr. 2013;62(1):28–35.
47. Van Praag RME, van Weert ECM, van Heeswijk RPG, et al. Stable concentrations of zidovudine, stavudine, lamivudine, abacavir, and nevirapine in serum and cerebrospinal fluid during 2 years of therapy. Antimicrob Agents Chemother. 2002;46(3):896–9.
48. Saitoh A, Sarles E, Capparelli E, et al. CYP2B6 genetic variants are associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. AIDS. 2007;21(16):2191–9.
49. Antinori A, Perno CF, Giancola ML, et al. Efficacy of cerebrospinal fluid (CSF)-penetrating antiretroviral drugs against HIV in the neurological compartment: different patterns of phenotypic resistance in CSF and plasma. Clin Infect Dis. 2005;41(12):1787–93.
50. Nguyen A, Rossi S, Croteau D, et al. Etravirine in CSF is highly protein bound. J Antimicrob Chemother. 2013;68(5):1161–8.
51. Tiraboschi JM, Niubo J, Vila A, Perez-Pujol S, Podzamczer D. Etravirine concentrations in CSF in HIV-infected patients. J Antimicrob Chemother. 2012;67(6):1446–8.
52. Haas DW, Johnson B, Nicotera J, et al. Effects of ritonavir on indinavir pharmacokinetics in cerebrospinal fluid and plasma. Antimicrob Agents Chemother. 2003;47(7):2131–7.
53. Croteau D, Rossi SS, Best BM, et al. Darunavir is predominantly unbound to protein in cerebrospinal fluid and concentrations exceed the wild-type HIV-1 median 90 % inhibitory concentration. J Antimicrob Chemother. 2013;68(3):684–9.
54. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. 2014 [Table 8, page 25]. http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed 21 Oct 2014.
55. Van Praag RM, Weverling GJ, Portegies P, et al. Enhanced penetration of indinavir in cerebrospinal fluid and semen after the addition of low-dose ritonavir. AIDS. 2000;14(9):1187–94.
56. Letendre SL, Capparelli EV, Ellis RJ, McCutchan JA. Indinavir population pharmacokinetics in plasma and cerebrospinal fluid. The HIV Neurobehavioral Research Center Group. Antimicrob Agents Chemother. 2000;44(8):2173–5.
57. Polis MA, Suzman DL, Yoder CP, et al. Suppression of cerebrospinal fluid HIV burden in antiretroviral naive patients on a potent four-drug antiretroviral regimen. AIDS. 2003;17(8):1167–72.
58. Kalvass JC, Polli JW, Bourdet DL, et al. Why clinical modulation of efflux transport at the human blood-brain barrier is unlikely: the ITC evidence-based position. Clin Pharmacol Ther. 2013;94(1):80–94.
59. Best BM, Letendre SL, Brigid E, et al. Low atazanavir concentrations in cerebrospinal fluid. AIDS. 2009;23(1):83–7.
60. Lafeuillade A, Solas C, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Differences in the detection of three HIV-1 protease inhibitors in non-blood compartments: clinical correlations. HIV Clin Trials. 2002;3(1):27–35.
61. Solas C, Lafeuillade A, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. Antimicrob Agents Chemother. 2003;47(1):238–43.
62. Aweeka F, Jayewardene A, Staprans S, et al. Failure to detect nelfinavir in the cerebrospinal fluid of HIV-1–infected patients with and without AIDS dementia complex. J Acquir Immune Defic Syndr Hum Retrovirol. 1999;20(1):39–43.
63. Yilmaz A, Fuchs D, Hagberg L, et al. Cerebrospinal fluid HIV-1 RNA, intrathecal immunoactivation, and drug concentrations after treatment with a combination of saquinavir, nelfinavir, and two nucleoside analogues: the M61022 study. BMC Infect Dis. 2006;6:63.
64. Karlström O, Ståhle L, Perrin L, Tegude H, Sönnerborg A. Efficacy of nelfinavir-based treatment in the central nervous system of HIV-1 infected patients. Scand J Infect Dis. 2006;38(5):371–4.
65. Capparelli EV, Holland D, Okamoto C, et al. Lopinavir concentrations in cerebrospinal fluid exceed the 50 % inhibitory concentration for HIV. AIDS. 2005;19(9):949–52.
66. DiCenzo R, DiFrancesco R, Cruttenden K, Donnelly J, Schifitto G. Lopinavir cerebrospinal fluid steady-state trough concentrations in HIV-infected adults. Ann Pharmacother. 2009;43(12):1972–7.
67. Yilmaz A, Izadkhashti A, Price RW, et al. Darunavir concentrations in cerebrospinal fluid and blood in HIV-1-infected individuals. AIDS Res Hum Retrovir. 2009;25(4):457–61.
68. Calcagno A, Yilmaz A, Cusato J, et al. Determinants of darunavir cerebrospinal fluid concentrations: impact of once-daily dosing and pharmacogenetics. AIDS. 2012;26(12):1529–33.
69. Khaliq Y, Gallicano K, Venance S, Kravcik S, Cameron DW. Effect of ketoconazole on ritonavir and saquinavir concentrations in plasma and cerebrospinal fluid from patients infected with human immunodeficiency virus. Clin Pharmacol Ther. 2000;68(6):637–46.
70. Moyle GJ, Sadler M, Buss N. Plasma and cerebrospinal fluid saquinavir concentrations in patients receiving combination antiretroviral therapy. Clin Infect Dis. 1999;28:403–4.
71. Kravcik S, Gallicano K, Roth V, et al. Cerebrospinal fluid HIV RNA and drug levels with combination ritonavir and saquinavir. J Acquir Immune Defic Syndr. 1999;21(5):371–5.
72. Price RW, Parham R, Kroll JL, et al. Enfuvirtide cerebrospinal fluid (CSF) pharmacokinetics and potential use in defining CSF HIV-1 origin. Antivir Ther. 2008;13(3):369–74.

73. Yilmaz A, Watson V, Else L, Gisslèn M. Cerebrospinal fluid maraviroc concentrations in HIV-1 infected patients. AIDS. 2009;23(18):2537–40.
74. Yilmaz A, Gisslén M, Spudich S, et al. Raltegravir cerebrospinal fluid concentrations in HIV-1 infection. PLoS One. 2009;4(9): e6877.
75. Johnson DH, Sutherland D, Acosta EP, Erdem H, Richardson D, Haas DW. Genetic and non-genetic determinants of raltegravir penetration into cerebrospinal fluid: a single arm pharmacokinetic study. PLoS One. 2013;8(12):5156–60.
76. Eilers M, Roy U, Mondal D. MRP (ABCC) transporters-mediated efflux of anti-HIV drugs, saquinavir and zidovudine, from human endothelial cells. Exp Biol Med (Maywood). 2008;233(9): 1149–60.
77. Cordon-Cardo C, O'Brien JP, Casals D, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci. 1989;86(2):695–8.
78. Kusuhara H, Sugiyama Y. Active efflux across the blood-brain barrier: role of the solute carrier family. NeuroRx. 2005;2(1): 73–85.
79. Kim RB. Drug transporters in HIV therapy. Top HIV Med. 2003;11(4):136–9.
80. Johnson DH, Sutherland D, Acosta EP, Erdem H, Richardson D, Haas DW. Genetic and non-genetic determinants of raltegravir penetration into cerebrospinal fluid: a single arm pharmacokinetic study. PLoS One. 2013;8(12):e82672.
81. Haas DW, Clough LA, Johnson BW, et al. Evidence of a source of HIV type 1 within the central nervous system by ultraintensive sampling of cerebrospinal fluid and plasma. AIDS Res Hum Retrovir. 2000;16(15):1491–502.
82. Yasuda SU, Zhang L, Huang SM. The role of ethnicity in variability in response to drugs: focus on clinical pharmacology studies. Clin Pharmacol Ther. 2008;84(3):417–23.
83. Capparelli EV, Letendre SL, Ellis RJ, Patel P, Holland D, Mccutchan JA. Population pharmacokinetics of abacavir in plasma and cerebrospinal fluid. Antimicrob Agents Chemother. 2005; 49(6):2504–6.
84. Van Leeuwen R, Katlama C, Kitchen V, et al. Evaluation of safety and efficacy of 3TC (lamivudine) in patients with asymptomatic or mildly symptomatic human immunodeficiency virus infection: a phase I/II study. J Infect Dis. 1995;171(5): 1166–71.
85. Mueller BU, Lewis LL, Yuen GJ, et al. Serum and cerebrospinal fluid pharmacokinetics of intravenous and oral lamivudine in human immunodeficiency virus-infected children. Antimicrob Agents Chemother. 1998;42(12):3187–92.
86. Foudraine NA, Hoetelmans RM, Lange JM, et al. Cerebrospinal-fluid HIV-1 RNA and drug concentrations after treatment with lamivudine plus zidovudine or stavudine. Lancet. 1998;351(9115): 1547–51.
87. Haworth SJ, Christofalo B, Anderson RD, Dunkle LM. A single-dose study to assess the penetration of stavudine into human cerebrospinal fluid in adults. J Acquir Immune Defic Syndr Hum Retrovirol. 1998;17(3):235–8.
88. Hoetelmans RM, Kraaijeveld CL, Meenhorst PL, et al. Penetration of 3′-amino-3′-deoxythymidine, a cytotoxic metabolite of zidovudine, into the cerebrospinal fluid of HIV-1-infected patients. J Acquir Immune Defic Syndr Hum Retrovirol. 1997;15(2):131–6.
89. Martin C, Sönnerborg A, Svensson JO, Ståhle L. Indinavir-based treatment of HIV-1 infected patients: efficacy in the central nervous system. AIDS. 1999;13(10):1227–32.
90. Zhou XJ, Havlir DV, Richman DD, et al. Plasma population pharmacokinetics and penetration into cerebrospinal fluid of indinavir in combination with zidovudine and lamivudine in HIV-1-infected patients. AIDS. 2000;14(18):2869–76.
91. Vernazza P, Daneel S, Schiffer V, et al. The role of compartment penetration in PI-monotherapy: the Atazanavir-Ritonavir Monomaintenance (ATARITMO) Trial. AIDS. 2007;21(10):1309–15.
92. Croteau D, Letendre S, Best BM, et al. Total raltegravir concentrations in cerebrospinal fluid exceed the 50-percent inhibitory concentration for wild-type HIV-1. Antimicrob Agents Chemother. 2010;54(12):5156–60.

CHAPTER 3

Neuronal toxicity of efavirenz: a systematic review.

EXPERT OPINION

Neuronal toxicity of efavirenz: a systematic review

Eric H Decloedt† & Gary Maartens
†*Stellenbosch University, Faculty of Medicine and Health Sciences, Division of Clinical Pharmacology, Department of Medicine, Tygerberg, South Africa*

Introduction: Efavirenz commonly causes early neuropsychiatric side effects, but tolerance develops in most patients. There is emerging evidence that efavirenz use may damage neurons, which could result in impaired neurocognitive performance.

Areas covered: The authors conducted a systematic review using the PubMed database, references cited by other articles and conference web sites to determine if there is evidence that efavirenz may contribute to cognitive impairment by damaging nerve cells.

Expert opinion: There is weak clinical evidence suggesting that efavirenz use may worsen neurocognitive impairment or be associated with less improvement in neurocognitive impairment than other antiretrovirals. Efavirenz, especially its major metabolite 8-hydroxy-efavirenz, is toxic in neuron cultures at concentrations found in the cerebrospinal fluid. Extensive metabolizers of efavirenz may therefore be more likely to develop efavirenz toxicity by forming more 8-hydroxy-efavirenz. Several potential mechanisms exist to explain the observed efavirenz neurotoxicity, including altered calcium hemostasis, decreases in brain creatine kinase, mitochondrial damage, increases in brain proinflammatory cytokines and involvement of the cannabinoid system. There is a need for large randomized controlled trials to determine if the neuronal toxicity induced by efavirenz results in clinically significant neurological impairment.

Keywords: efavirenz, neurocognitive impairment, neurotoxicity

Expert Opin. Drug Saf. (2013) 12(6):841-846

1. Introduction

Efavirenz is a widely used non-nucleoside reverse transcriptase inhibitor in combination with other antiretroviral agents for the treatment of human immunodeficiency virus type 1 (HIV-1) infection. Efavirenz use is commonly complicated by early neuropsychiatric adverse events in up to 68% of patients [1-5]. Most patients report mild symptoms of headache, dizziness, impaired concentration, sleep disturbance, abnormal dreams, anxiety or depression [2]. Clinical symptoms commonly start within days, but even after a single dose of efavirenz, the neuropsychiatric testing domains are affected [1,6,7]. Symptoms peak at 1 week after treatment initiation and usually resolve within the first month despite ongoing ingestion of efavirenz. One study reported that mild neuropsychiatric symptoms persisted for up to 3 years in many patients [8]. Early severe neuropsychiatric symptoms usually resolve on discontinuation of efavirenz [9-14]. A recent case report of vacuolar axonopathy leading to a depressed level of consciousness, aspiration pneumonia and death was thought to be efavirenz induced, suggesting that efavirenz neurotoxicity may on occasion be more severe than transient neuropsychiatric effects [15].

There is emerging evidence that efavirenz use may damage neurons, which could result in impaired neurocognitive performance. We conducted a systematic review using the electronic journal database PubMed, Cochrane Database of Systematic

10.1517/14740338.2013.823396 © 2013 Informa UK, Ltd. ISSN 1474-0338, e-ISSN 1744-764X

Article highlights.

- Efavirenz commonly causes early neuropsychiatric side effects, but tolerance develops in most patients.
- There is emerging clinical evidence indicating that efavirenz use may worsen neurocognitive impairment or be associated with less improvement in neurocognitive impairment than other antiretrovirals.
- Efavirenz and especially the major metabolite 8-hydroxy-efavirenz are toxic in neuron cultures at concentrations found in the CSF.
- There is a need for large randomized controlled trials to determine if the neuronal toxicity induced by efavirenz results in clinically significant neurological impairment before any conclusions can be made about ongoing use of this widely used antiretroviral drug.

This box summarizes key points contained in the article.

Reviews and Google Scholar. We conducted our search from 1997 onward to capture all studies from 1998 when efavirenz was approved by the US Food and Drug Agency (FDA). We also searched the electronic conference databases of the International Workshop on Clinical Pharmacology of HIV Therapy, Conferences on Retroviruses and Opportunistic Infections and International AIDS Conference. Our search strategy included but was not limited to the following medical subject heading (MeSH) terms: efavirenz, toxicity, AIDS dementia complex, neurons, neuropsychiatric disorders and neurocognitive impairment. We included all fields in our search but limited our search to the English literature. We also scrutinized the citations of reviewed articles for any references not identified in our search.

Several studies have shown a correlation between higher plasma efavirenz concentrations and an increased risk of early neuropsychiatric side effects [8,16-21]. Efavirenz concentrations are characterized by marked interpatient variability, some of which is explained by polymorphisms in CYP2B6, the major cytochrome P450 metabolizing enzyme [22]. Efavirenz is metabolized into three metabolites: two hydroxylated metabolites 8-hydroxy-efavirenz and 7-hydroxy-efavirenz and a third metabolite that undergoes glucuronidation. More than 90% of efavirenz is metabolized by CYP2B6 into the major metabolite 8-hydroxy-efavirenz. CYP2A6 metabolizes < 8% of efavirenz into 7-hydroxy-efavirenz [23]. UGT2B7 is responsible for conjugation to efavirenz-glucuronide [24]. Three polymorphisms in CYP2B6 are associated with higher efavirenz concentrations [22]. The CYP2B6 516 G→T polymorphism has the strongest effect on efavirenz concentrations, followed by the 983 T→C polymorphism [22]. An *in vitro–in vivo* extrapolation model estimated that a dose reduction from 600 to 400 mg in patients with the 516 GT genotype and to 200 mg in those with the 516 TT genotype could reduce the risk of developing early neuropsychiatric side effects without affecting the probability of viral suppression [21].

Efavirenz plasma drug concentrations correlate with central nervous system (CNS) drug concentrations. The brain is protected from transient changes in the composition of the blood by the blood–brain barrier (BBB) and the blood–cerebrospinal fluid (CSF) barrier (BCSFB). The BBB and BCSFB are not passive anatomical barriers but dynamic interfaces that express a variety of influx and efflux transporters. Drug influx and efflux transporters in the BBB play an important role in the disposition of drugs in the CNS and may impede access of antiretroviral drugs to the CNS [25,26]. Transporters at the BBB and the BCSFB that have been implicated in the penetration of antiretrovirals into the CNS includes *p*-glycoprotein (MDR-1 or ABCB1), organic cation transporters (OCTs), organic anion transporters (OATs), organic anion-transporting polypeptide (OATP), breast cancer resistance protein (BRCP) and multidrug resistance-associated proteins (MRP or ABCC) [25,27,28]. There is weak evidence that efavirenz is a substrate of *p*-glycoprotein, which is an important BBB efflux pump [29-31]. However, unbound efavirenz concentrations in the plasma and CSF have recently been shown to be similar in humans despite large differences in the total CSF and plasma efavirenz concentrations, suggesting that simple diffusion rather than transporter-mediated efflux or influx determines efavirenz CSF concentrations [32].

There is emerging clinical evidence indicating that efavirenz use may worsen neurocognitive impairment or be associated with less improvement in neurocognitive impairment than other antiretrovirals. A small open-label study randomly assigned HIV-infected patients commencing antiretroviral therapy (ART) of tenofovir plus emtricitabine combined with either efavirenz (n = 9), atazanavir plus ritonavir (n = 8) or zidovudine plus abacavir (n = 11) [33]. Changes in neurocognitive function were assessed at baseline, week 24 and week 48 using a validated battery of tests. Patients receiving the efavirenz-based regimen had less overall neurocognitive improvement and performed statistically significantly worse compared with the other two arms on the speed domains on follow-up neuropsychiatric testing. The potential neurotoxicity of ART was explored in a cohort of asymptomatic HIV-infected patients on ART [34]. Twenty-five out of the 129 (19%) patients on ART received efavirenz-based ART. In multivariate analysis, efavirenz use independently predicted worse performance in tests of higher attention and executive load domains. The association remained significant when the analysis was restricted to patients on their current ART regimen for 1 year or longer. ART improves HIV-associated neurocognitive disorder, but residual impairment is common [35,36]. Therefore, one would expect that neurocognition would worsen when ART is discontinued. However, a cohort study of patients who had been on ART for a median of 4.5 years with CD4 counts > 350 cells/mm^3 who elected to interrupt ART found that scores of neuropsychological tests actually improved after discontinuing ART [37]. Interruption of efavirenz-based ART was associated with greater neurocognitive improvement compared with other regimens.

There is evidence to suggest that efavirenz is directly toxic to neurons in laboratory studies. Two recent studies evaluated

the direct effect of efavirenz on cultures of rat neurons [38,39]. In the first study, direct neurotoxicity of 15 antiretroviral drugs, including efavirenz was evaluated [38]. The neuron cultures were challenged for 1 week with each of the antiretroviral drugs at a range of concentrations. The antiretroviral drug concentrations ranged from at least 1 order of magnitude above and below the therapeutic plasma concentrations. Neuronal damage was quantified using microtubule-associated protein-2 (MAP-2) immunostaining, measuring the loss of area occupied by MAP-2-stained neurons. A toxicity index was calculated using toxicity data derived from the dose–response curves for neuronal damage as well as the therapeutic antiretroviral drug plasma concentrations and the estimated CSF concentrations. Highest neurotoxicities were seen with abacavir, atazanavir, efavirenz, etravirine and nevirapine at drug concentrations similar to plasma concentrations. However, estimated neurotoxicity risk of efavirenz toxicity was low with predicted CSF concentrations. In the second study, the effect of efavirenz and its metabolites on calcium homeostasis, dendritic spine morphology and survival of rat neuronal cultures was studied [39]. 8-Hydroxy-efavirenz, but not efavirenz or 7-hydroxy-efavirenz, caused a loss of membrane integrity and release of calcium. Although both efavirenz and its metabolites induced neuronal damage in a dose-dependent manner, 8-hydroxy-efavirenz was 10-fold more toxic. The investigators measured CSF efavirenz and 8-hydroxy-efavirenz concentrations from 13 patients established on efavirenz-based ART enrolled on a neurocognitive impairment study. CSF concentrations of both efavirenz and 8-hydroxy-efavirenz were found to be similar to the concentrations to elicited neuronal damage in the rat neurons.

Several potential mechanisms exist to explain efavirenz neurotoxicity. First, efavirenz and its metabolites may cause direct neuronal damage by disrupting calcium homeostasis [39]. Second, creatine kinase (CK) concentrations were significantly reduced in the cortex and cerebellum in mice treated with efavirenz [40]. A decrease in CK has been associated with neurodegenerative diseases [41]. Third, efavirenz causes a concentration-dependent mitochondriopathy in human hepatic cells by inhibiting complex 1 of the respirator chain [42]. Mitochondria play a crucial role and cell survival and a decline in mitochondrial function has been associated with aging and dementia [43]. Efavirenz reduces mitochondrial respiratory chain complex IV activity in the brains of mice [44]. However, neurotoxicity was unrelated to mitochondrial damage or cell death in rat neurons [38,44]. Fourth, efavirenz-treated rats displayed memory deficits and stress, which were associated with increased proinflammatory cytokines interleukin-1 beta and tumor necrosis factor-alpha [45]. Cytokines and their signaling pathways affect the metabolism of multiple neurotransmitters such as serotonin, dopamine and glutamate by altering their synthesis, release and reuptake [46]. Finally, efavirenz is selective cytotoxic against cancer cells. The antineoplastic effect of efavirenz is possibly mediated via cannabinoid signaling pathways [47].

The weak evidence that efavirenz may worsen HIV-associated neurocognitive impairment should be viewed in context of the high risk of severe AIDS dementia with untreated HIV infection. Efavirenz is widely recommended as a first-line ART in both resource-rich and resource-poor settings because it is well tolerated and effective [48,49]. In low- and middle-income countries, HIV-associated tuberculosis is very common; therefore, the fact that efavirenz-based ART is less prone to interactions with antituberculosis therapy compared with other ART is an important advantage [50].

2. Conclusion

Efavirenz commonly causes transient early neuropsychiatric side effects. There is emerging clinical evidence, indicating that efavirenz use may worsen neurocognitive impairment or be associated with less improvement in neurocognitive impairment than other antiretrovirals. Animal data indicate that efavirenz, especially its major metabolite 8-hydroxy-efavirenz, is directly toxic to neurons at concentrations achieved in the CSF of HIV-infected patients. Several potential mechanisms exist to explain efavirenz neurotoxicity, including altered calcium haemostasis, decreases in brain CK, mitochondrial damage, increases in proinflammatory cytokines and involvement of the cannabinoid system. There is a need for randomized controlled trials to determine if the neuronal toxicity induced by efavirenz results in a clinically significant neurological impairment. Until these trials are conducted, it is premature to recommend against the use of efavirenz.

3. Expert opinion

Efavirenz commonly causes mild neuropsychiatric effects of therapy, but tolerance develops over a few weeks, even in patients with CYP2B6 polymorphisms resulting in higher efavirenz concentrations [51]. These transient early neuropsychiatric side effects do not appear to cause permanent damage and seldom result in discontinuation. However, if efavirenz use is associated with an increased risk of neurocognitive impairment, this could result in significant disability.

The clinical evidence that efavirenz use may worsen neurocognitive impairment or be associated with less improvement in neurocognitive impairment than other antiretrovirals is weak. Animal data indicate that efavirenz is neurotoxic, together with a number of other antiretrovirals. A key finding in our understanding of efavirenz neurotoxicity is that the 8-hydroxy-efavirenz is 10 times more toxic in neuron cultures compared with the parent drug. The implication for this finding is that extensive metabolizers, who generate more 8-hydroxy-efavirenz, may develop more neurotoxicity. A number of mechanisms could account for the observed neurotoxicity, either acting singly or in combination. One of the plausible mechanisms is that efavirenz acts indirectly on the cannabinoid system or its downstream signaling mechanisms.

The action of efavirenz on the cannabinoid system may explain the resemblance with the side-effect profile of orally administered tetrahydrocannabinol such as dizziness, somnolence and abnormal dreams [47]. There is limited evidence currently to suggest that efavirenz directly influences neurotransmitter pathways.

Inferences about efavirenz neurotoxicity are usually made using plasma concentrations. A therapeutic window for ART effectiveness in the CNS probably exists, but the therapeutic range is poorly defined [39,52]. In the majority of studies, a simultaneous single CSF and plasma concentration is measured and expressed as a ratio. Single-point CSF:plasma ratios are less accurate approximations of drug exposure as plasma concentrations vary more widely over time compared with CSF concentrations [53]. The ratio of the average steady-state unbound plasma:CSF concentrations is the gold standard and is approximated by the area under the concentration–time curves (AUC) for CSF and plasma [53,54]. However, frequent CSF sampling to determine AUC is generally not feasible. Future approaches could use population pharmacokinetic modeling to estimate pharmacokinetic parameters from a single sample collected at different times from multiple patients to estimate the plasma:CSF exposure [55-57].

The next step would be to conduct an adequately powered clinical trial to determine if the neurotoxicity of efavirenz results in inferior neurologic outcomes that are clinically significant. Determining the genotypic metabolizer status and plasma concentrations of efavirenz and the 8-hydroxy metabolite would be important variables to measure in the clinical trial, which would further our understanding of neurotoxicity. It is important that unbound efavirenz CSF drug concentrations are measured since only the unbound drug fraction crosses the BBB. CSF analysis should include the 8-hydroxy metabolite in addition to efavirenz concentrations. Plasma and CSF efavirenz and metabolite concentrations as well as genotypic metabolizer status should be used as variables to develop an algorithm that permits safer use of this widely used antiretroviral drug.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Kenedi CA, Goforth HW. A systematic review of the psychiatric side-effects of efavirenz. AIDS Behav 2011;15(8):1803-18
2. Sustive I(efavirenz) package insert [Internet]. 2012. Available from: http://packageinserts.bms.com/pi/pi_sustiva.pdf
3. Staszewski S, Morales-Ramirez J, Tashima KT, et al. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 team. N Engl J Med 1999;341(25):1865-73
4. Cavalcante GI, Capistrano VL, Cavalcante FS, et al. Implications of efavirenz for neuropsychiatry: a review. Int J Neurosci 2010;120(12):739-45
5. Cespedes MS, Aberg JA. Neuropsychiatric complications of antiretroviral therapy. Drug Saf 2006;29(10):865-74
6. Johnson DH, Gebretsadik T, Shintani A, et al. Neuropsychometric correlates of efavirenz pharmacokinetics and pharmacogenetics following a single oral dose. Br J Clin Pharmacol 2013;75(4):997-1006
7. de la Garza CL, Paoletti-Duarte S, Garcia-Martin C, et al. Efavirenz-induced psychosis. AIDS 2001;15(14):1911-12
8. Clifford DB, Evans S, Yang Y, et al. Long-term impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals (ACTG 5097s). HIV Clin Trials 2009;10(6):343-55
9. Manning TG. Efavirenz and psychosis: is there a link? Aust NZ J Psychiatry 2012;46(7):687-8
10. Zalila H, Elloumi H, Gaha N, et al. Acute psychosis under efavirenz in a HIV patient. Tunis Med 2010;88(2):119-21
11. Salter E, Patel AS. Neuropsychiatric sequelae in an efavirenz treated patient with hepatitis B. BMJ Case Rep 2009; Epub ahead of print
12. Poulsen HD, Lublin HK. Efavirenz-induced psychosis leading to involuntary detention. AIDS 2003;17(3):451-3
13. Blanch J, Corbella B, Garcia F, et al. Manic syndrome associated with efavirenz overdose. Clin Infect Dis 2001;33(2):270-1
14. Shah MD, Balderson K. A manic episode associated with efavirenz therapy for HIV infection. AIDS 2003;17(11):1713-14
15. Kenyon C, Mfolozi S, Croxford R, et al. Severe efavirenz-induced vacuolar axonopathy complicated by fatal aspiration pneumonia. Br J Clin Pharmacol 2012;74(6):1070-2
16. van Luin M, Gras L, Richter C, et al. Efavirenz dose reduction is safe in patients with high plasma concentrations and may prevent efavirenz discontinuations. J Acquir Immune Defic Syndr 2009;52(2):240-5
17. Marzolini C, Telenti A, Decosterd LA, et al. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS 2001;15(1):71-5
18. Gutierrez F, Navarro A, Padilla S, et al. Prediction of neuropsychiatric adverse events associated with long-term efavirenz therapy, using plasma drug level monitoring. Clin Infect Dis 2005;41(11):1648-53
19. Nunez M, Gonzalez de Requena D, Gallego L, et al. Higher efavirenz plasma levels correlate with development of insomnia. J Acquir Immune Defic Syndr 2001;28(4):399-400
20. Clifford DB, Evans S, Yang Y, et al. Impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals. Ann Intern Med 2005;143(10):714-21

21. Siccardi M, Almond L, Schipani A, et al. Pharmacokinetic and pharmacodynamic analysis of efavirenz dose reduction using an in vitro-in vivo extrapolation model. Clin Pharmacol Ther 2012;92(4):494-502

• **An *in vitro-in vivo* extrapolation model estimating that an efavirenz dose reduction in patients with the CYP2B6 516 G→T could reduce the risk of patients developing early neuropsychiatric side effects.**

22. Holzinger ER, Grady B, Ritchie MD, et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS clinical trials group protocols implicates several CYP2B6 variants. Pharmacogenet Genomics 2012;22(12):858-67

•• **The first genome-wide association analyses of efavirenz pharmacokinetics.**

23. di Iulio J, Fayet A, Arab-Alameddine M, et al. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. Pharmacogenet Genomics 2009;19(4):300-9

24. Belanger AS, Caron P, Harvey M, et al. Glucuronidation of the antiretroviral drug efavirenz by UGT2B7 and an in vitro investigation of drug-drug interaction with zidovudine. Drug Metab Dispos 2009;37(9):1793-6

25. Minuesa G, Huber-Ruano I, Pastor-Anglada M, et al. Drug uptake transporters in antiretroviral therapy. Pharmacol Ther 2011;132(3):268-79

26. Eyal S, Hsiao P, Unadkat JD. Drug interactions at the blood-brain barrier: fact or fantasy? Pharmacol Ther 2009;123(1):80-104

27. Eilers M, Roy U, Mondal D. MRP (ABCC) transporters-mediated efflux of anti-HIV drugs, saquinavir and zidovudine, from human endothelial cells. Exp Biol Med (Maywood) 2008;233(9):1149-60

28. Varatharajan L, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary of current knowledge and recommendations for further research. Antiviral Res 2009;82(2):A99-109

29. Haas DW, Smeaton LM, Shafer RW, et al. Pharmacogenetics of long-term responses to antiretroviral regimens containing efavirenz and/or nelfinavir: an adult aids clinical trials group study. J Infect Dis 2005;192(11):1931-42

30. Elens L, Vandercam B, Yombi JC, et al. Influence of host genetic factors on efavirenz plasma and intracellular pharmacokinetics in HIV-1-infected patients. Pharmacogenomics 2010;11(9):1223-34

31. Swart M, Ren Y, Smith P, et al. ABCB1 4036A>G and 1236C>T polymorphisms affect plasma efavirenz levels in South African HIV/AIDS patients. Front Genet 2012;3:236

32. Avery LB, Sacktor N, McArthur JC, et al. Protein-free efavirenz concentrations in cerebrospinal fluid and blood plasma are equivalent: applying the law of mass action to predict protein-free drug concentration. Antimicrob Agents Chemother 2013;57(3):1409-14

•• **An important study showing that the unbound efavirenz CSF concentrations are similar to the unbound efavirenz plasma concentrations.**

33. Winston A, Puls R, Kerr SJ, et al. Dynamics of cognitive change in HIV-infected individuals commencing three different initial antiretroviral regimens: a randomized, controlled study. HIV Med 2012;13(4):245-51

• **Clinical evidence suggesting that efavirenz use may worsen neurocognitive impairment.**

34. Ciccarelli N, Fabbiani M, Di Giambenedetto S, et al. Efavirenz associated with cognitive disorders in otherwise asymptomatic HIV-infected patients. Neurology 2011;76(16):1403-9

• **Clinical evidence suggesting that efavirenz use may worsen neurocognitive impairment.**

35. Heaton RK, Franklin DR, Ellis RJ, et al. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. J Neurovirol 2011;17(1):3-16

36. Heaton RK, Clifford DB, Franklin DR Jr, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER study. Neurology 2010;75(23):2087-96

37. Robertson KR, Su Z, Margolis DM, et al. Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. Neurology 2010;74(16):1260-6

38. Robertson K, Liner J, Meeker RB. Antiretroviral neurotoxicity. J Neurovirol 2012;18(5):388-99

•• **Animal data suggesting that efavirenz as well as other antiretroviral therapy are neurotoxic at estimated doses that reach the CNS.**

39. Tovar-y-Romo LB, Bumpus NN, Pomerantz D, et al. Dendritic spine injury induced by the 8-hydroxy metabolite of efavirenz. J Pharmacol Exp Ther 2012;343(3):696-703

•• **Animal data suggesting that the 8-hydroxy metabolite of efavirenz is ten times more neurotoxic than efavirenz.**

40. Streck EL, Scaini G, Rezin GT, et al. Effects of the HIV treatment drugs nevirapine and efavirenz on brain creatine kinase activity. Metab Brain Dis 2008;23(4):485-92

41. Zhang SF, Hennessey T, Yang L, et al. Impaired brain creatine kinase activity in huntington's disease. Neurodegener Dis 2011;8(4):194-201

42. Blas-Garcia A, Apostolova N, Ballesteros D, et al. Inhibition of mitochondrial function by efavirenz increases lipid content in hepatic cells. Hepatology 2010;52(1):115-25

43. Swerdlow RH. Brain aging, alzheimer's disease, and mitochondria. Biochim Biophys Acta 2011;1812(12):1630-9

44. Streck EL, Ferreira GK, Scaini G, et al. Non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine inhibit cytochrome C oxidase in mouse brain regions. Neurochem Res 2011;36(6):962-6

45. O'Mahony SM, Myint AM, Steinbusch H, et al. Efavirenz induces depressive-like behaviour, increased stress response and changes in the immune response in rats. Neuroimmunomodulation 2005;12(5):293-8

46. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. Depress Anxiety 2013;30(4):297-306

47. Hecht M, Harrer T, Buttner M, et al. Cytotoxic effect of efavirenz is selective against cancer cells and associated with

the cannabinoid system. AIDS 2013. [Epub ahead of print]

•• **In vivo data suggesting that efavirenz antitumor activity is mediated indirectly via the cannabinoid system.**

48. Thompson MA, Aberg JA, Hoy JF, et al. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the international antiviral society-USA panel. JAMA 2012;308(4):387-402

49. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach. 2010. Available from: http://www.who.int/hiv/pub/arv/adult2010/en/index.html

50. Maartens G, Decloedt E, Cohen K. Effectiveness and safety of antiretrovirals with rifampicin: crucial issues for high-burden countries. Antivir Ther 2009;14(8):1039-43

51. Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an adult AIDS clinical trials group study. AIDS 2004;18(18):2391-400

52. Letendre S. Central nervous system complications in HIV disease: HIV-associated neurocognitive disorder. Top Antivir Med 2011;19(4):137-42

53. Enting RH, Hoetelmans RM, Lange JM, et al. Antiretroviral drugs and the central nervous system. AIDS 1998;12(15):1941-55

54. Wynn HE, Brundage RC, Fletcher CV. Clinical implications of CNS penetration of antiretroviral drugs. CNS Drugs 2002;16(9):595-609

55. Letendre SL, Capparelli EV, Ellis RJ, et al. Indinavir population pharmacokinetics in plasma and cerebrospinal fluid. the HIV neurobehavioral research center group. Antimicrob Agents Chemother 2000;44(8):2173-5

56. Capparelli EV, Letendre SL, Ellis RJ, et al. Population pharmacokinetics of abacavir in plasma and cerebrospinal fluid. Antimicrob Agents Chemother 2005;49(6):2504-6

57. Zhou XJ, Havlir DV, Richman DD, et al. Plasma population pharmacokinetics and penetration into cerebrospinal fluid of indinavir in combination with zidovudine and lamivudine in HIV-1-infected patients. AIDS 2000;14(18):2869-76

Affiliation

Eric H Decloedt[†1] MBChB BSc Hons (Pharmacology) FCCP (SA) MMed (Clin Pharm) &
Gary Maartens[2] MBChB MMed (Int Med) FCP (SA) DTM&H
[†]Author for correspondence
[1]Clinical Pharmacologist,
Stellenbosch University,
Faculty of Medicine and Health Sciences,
Division of Clinical Pharmacology,
Department of Medicine, PO Box 19063;
Francie van Zijl Drive, Tygerberg 7505,
South Africa
E-mail: ericdecloedt@sun.ac.za
[2]University of Cape Town,
Faculty of Health Sciences,
Division of Clinical Pharmacology,
Department of Medicine,
K45, Old Main Building,
Groote Schuur Hospital, Observatory 7925,
South Africa

CHAPTER 4

Pharmacogenetics and pharmacokinetics of central nervous system penetration of efavirenz and its metabolites, tenofovir and emtricitabine.

Title

Pharmacogenetics and pharmacokinetics of central nervous system penetration of efavirenz and its metabolites, tenofovir and emtricitabine.

Short title

CNS PK and genomics of EFV, TFV and FTC

Author's names

Eric H. DECLOEDT[1,2], Phumla Z. SINXADI[2], Gert U VAN ZYL[4], Lubbe WIESNER[2], Saye KHOO[5], John A. JOSKA[3], David W. HAAS[6,7*], Gary MAARTENS[2*]

[*]These authors contributed equally

[1] Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

[2] Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, South Africa

[3] Division of Neuropsychiatry, Department of Psychiatry and Mental Health, University of Cape Town, South Africa

[4] Division of Medical Virology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, South Africa

[5] Department of Molecular and Clinical Pharmacology, University of Liverpool

[6] Vanderbilt University School of Medicine, Department of Medicine, Nashville, Tennessee, United States of America

[7] Meharry Medical College, Department of Internal Medicine, Nashville, Tennessee, United States of America

Corresponding author

Eric H DECLOEDT

Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa

ericdecloedt@sun.ac.za

Author contributions

Eric H. DECLOEDT: study concept and design, data acquisition, data analysis and interpretation of data, drafting and revising the manuscript for content, study supervision and obtaining funding

Phumla Z. SINXADI: data analysis, interpretation of data and revising the manuscript for content

Gert U. VAN ZYL: sample analysis, interpretation of data and revising the manuscript for content

Lubbe WIESNER: sample analysis and revising the manuscript for content

Saye KHOO: sample analysis and revising the manuscript for content

John A. JOSKA: study concept and design, revising the manuscript for content and study supervision

David W. HAAS: sample analysis, data analysis, interpretation of data and revising the manuscript for content

Gary MAARTENS: study concept and design and revising the manuscript for content

Synopsis

Background

There is limited data on pharmacogenetics and pharmacokinetics of central nervous system penetration of efavirenz, tenofovir and emtricitabine.

Objectives

We investigated genetic polymorphisms associated with cerebrospinal fluid (CSF) exposure of efavirenz and its metabolites, tenofovir and emtricitabine. The secondary objective was to explore the pharmacokinetic-pharmacodynamic relationships with neurocognitive performance.

Methods

We included 47 HIV-infected South African Black adults with and without HIV-associated neurocognitive disorder on antiretroviral therapy with efavirenz-tenofovir-emtricitabine for at least 6 months with suppressed viral loads. We collected paired plasma-CSF samples. We considered 2049 single-nucleotide polymorphisms (SNPs), including SNPs known to affect plasma efavirenz exposure, from potentially relevant genes (*ABCC5*, *ABCG2*, *ABCB1*, *SLCO2B1*, *SCLO1A2*, *ABCC4*, *CYP2B6* and *CYP2A6*) and 880 met a linkage disequilibrium (LD)-pruning threshold. We assessed neurocognitive impairment using Global Deficit Score.

Results

We identified 9 efavirenz slow, 21 intermediate and 17 extensive metabolizers based on composite *CYP2B6* 15582/516/983 genotype. A model that included composite *CYP2B6* 15582/516/983 genotype in univariate analyses best predicted $\log_{10}$-transformed concentrations of plasma efavirenz [β=0.34, 95% CI (0.20 to 0.48), *P*=1.7 x10^{-05}], plasma 7-hydroxy-efavirenz [β=0.45, 95% CI (0.10 to 0.66), *P*=5.8 x10^{-05}], plasma 8-hydroxy-efavirenz-to-efavirenz ratio [β=-0.29, 95% CI (-0.38 to -0.21), *P*=3.7 x10^{-08}] and CSF efavirenz [β=0.33, 95% CI (0.17 to 0.48), *P*=1.7 x10^{-05}]. Individual polymorphisms *CYP2B6* 516G→T and 983T→C also predicted these concentrations. Lower plasma 7-hydroxy-efavirenz concentrations were independently associated with *CYP2A6* rs10853742 [β=-0.55, 95% CI (-0.78 to -0.32), *P*=3.5 x10^{-05}],

ABCB1 rs115780656 [β=-0.65, 95% CI (-0.92 to -0.37), *P*=4.1 x10^{-05}] and *CYP2A6* -48A→C [β=-0.59, 95% CI (-1.01 to -0.16), *P*=1.0 x10^{-02}]. *CYP2A6* -48A→C was also independently associated with higher CSF 8-hydroxy-efavirenz-to-efavirenz ratio [β=0.55, 95% CI (0.05 to 1.03), *P*=4.8 x10^{-02}]. The *CYP2B6* rs2279345 polymorphism was associated with lower plasma 7-hydroxy-efavirenz-to-efavirenz ratio in univariate and multivariate analyses adjusting for *CYP2B6* 516G→T and 983T→C (*P*<0.05). No polymorphisms were associated with CSF-to-plasma ratios for each of the 3 drugs, plasma or CSF concentrations of 8-hydroxy-efavirenz, tenofovir or emtricitabine, or neurocognitive performance.

Conclusion

We identified novel genetic associations with plasma concentrations of efavirenz, 7-hydroxy-efavirenz, plasma 7-hydroxy-efavirenz-to-efavirenz ratio, plasma 8-hydroxy-efavirenz-to-efavirenz ratio, CSF efavirenz and CSF 8-hydroxy-efavirenz-to-efavirenz ratio concentrations.

Keywords

Pharmacokinetics, pharmacogenetics, efavirenz, tenofovir, emtricitabine, cerebrospinal fluid

Introduction

The fixed dose combination efavirenz (EFV), tenofovir (TFV) and emtricitabine (FTC) has been recommended by the World Health Organization (WHO) as first-line antiretroviral therapy (ART) for HIV-infected adults and is extensively prescribed.[1] However, prolonged ART exposure may cause a secondary decline in cognitive function when ART neurotoxicity exceeds central nervous system (CNS) viral suppression efficacy, a hypothesis supported by preclinical and limited clinical data.[2] Efavirenz in particular, but also TFV and FTC have been linked to direct neurotoxicity in *in vitro* studies individually and in combination.[3] Interrupting ART after a median of 4.5 years was associated with improved cognitive function, especially among EFV recipients.[4] In a randomised controlled trial (RCT), patients starting EFV, TFV and FTC rather than protease inhibitors or all-nucleoside reverse transcriptase inhibitor regimens had less improvement in neurocognitive function scores after 48 weeks.[5]

An antiretroviral CNS penetration-effectiveness (CPE) ranking has been developed, however it has not been conclusively shown that ART regimens with higher CPE result in improved cognitive function in patients with HIV-associated neurocognitive disorder (HAND).[6,7] However, higher CPE regimens have been associated with increased risk of HIV dementia, which could be due to antiretroviral toxicity.[8] Human genetic variants have been associated with antiretroviral pharmacokinetics and pharmacodynamics, but ART CNS-targeted strategies have not considered pharmacogenetics.[9] The impact of pharmacogenetics on the pharmacokinetics of EFV-TFV-FTC CNS penetration are lacking.

More than 90% of EFV is metabolized by cytochrome P450 (CYP) 2B6 into 8-hydroxy-EFV (8-OH-EFV) while CYP2A6 metabolism forms the 7-hydroxy-EFV (7-OH-EFV) metabolite.[10] The AIDS Clinical Trials Group first showed that the non-synonymous polymorphism *CYP2B6* 516G→T (rs3745274) was strongly associated with increased plasma EFV exposure and many studies have since replicated this association.[11–16] The *CYP2B6* 516 TT genotype is more common in Africans and African-Americans than in Caucasians, with a frequency of 11% to 23%.[11,17,18] Additional polymorphisms that are less frequent than 516G→T in Africans and African-Americans, 983T→C (rs28399499) and *CYP2B6* 15582C→T (rs4803419), also predict increased plasma EFV exposure.[10,18] The *CYP2B6* 983 C allele is found almost

exclusively with African ancestry with an allele frequency of only 4-9% but is associated with a 46% increase in plasma EFV concentrations.[18,19] Polymorphisms in genes beyond *CYP2B6* have been infrequently reported to be associated with EFV concentrations including polymorphisms in *CYP2A6.*[15,20] Polymorphisms in *CYP2B6* are associated with higher EFV plasma concentrations and predispose patients to EFV-mediated neurotoxicity.[11,21] Patients with *CYP2B6* slow metabolizer genotypes not only have higher plasma concentrations, but also higher cerebrospinal fluid (CSF) EFV exposure.[22] *In vitro* studies have implicated EFV and especially its metabolite 8-OH-EFV in causing direct neuronal toxicity.[3,23,24] In a CSF sub-study of the ENCORE1 trial, 8-OH-EFV exposure correlated with adverse neuropsychiatric outcomes.[22] However, the *CYP2B6* 516 G→T allele only predicted EFV plasma and CSF concentrations and not 8-OH-EFV plasma or CSF concentrations.[22,25,26]

Transporters expressed in the blood-brain barrier (BBB) and blood-CSF barrier (BCSFB) affect influx and efflux of drugs including antiretrovirals.[27,28] The superfamily of solute carriers (SLC) genes, including *SLCO2B1*, *SLCO1A2* and *SLCO1B1*, influence the expression of influx transporters in the BBB and BCSFB.[29,30] The efflux transporters in the BBB and BCSFB are influenced by adenosine triphosphate (ATP)-binding cassette (ABC) genes and include *ABCB1* (which encodes P-glycoprotein, P-gp), *ABCG2*, *ABCC4* and *ABCC5*.[30,31] Reported pharmacogenetic associations with EFV pharmacokinetics beyond *CYP2B6* and *CYP2A6* have not been well replicated. There is conflicting data regarding whether EFV is a P-gp substrate and whether *ABCB1* polymorphisms predict EFV concentrations.[13,32,33] *ABCC4* polymorphisms (rs1751034 and rs2274407) have been associated with lower and higher steady-state plasma EFV maximum concentrations respectively and in patients receiving EFV, *ABCG2* rs2231142 has been associated with an increased risk of abnormal dreams.[34,35] *ABCC5* transporters are ubiquitous and known are to mediate efflux of antiviral nucleotide analogs such as TFV and FTC.[36] *In vitro* assays have demonstrated that TFV is a substrate of *ABCG2*, *ABCC4* and P-gp, and that FTC is a substrate of *SLC47A1*.[37–39] TFV exposure in the CSF is approximately 6% of plasma concentrations and CSF penetration requires active transport.[40] *ABCG2* rs2231142 has been associated with a 1.5-fold increase in plasma TFV exposure.[41] Loss-of function polymorphisms in *ABCC4* may lead to reduced clearance of

TFV.[39] Antiretrovirals, mainly protease inhibitors, are also substrates of SLC transporters SLCO2B1, SLCO1A2 and SLCO1B1.[42]

Africans are the most genetically diverse population worldwide.[43] South Africa has the world's largest ART programme, with most patients currently receiving EFV-TFV-FTC.[44] Polymorphisms in genes that encode these enzymes or transporters may therefore affect EFV, TFV or FTC CSF penetration. We investigated associations between genetic polymorphisms and CSF exposure of EFV, 8-OH-EFV, TFV and FTC in Black South Africans. The secondary objective was to explore pharmacokinetic-pharmacodynamic relationships of CSF EFV, 8-OH-EFV, TFV and FTC with neurocognitive performance.

Patients and methods

Participants: Adults (≥ 18 and ≤ 70 years) who had participated in an RCT (PACTR201310000635418) investigating the safety and efficacy of lithium in patients with HIV-associated neurocognitive impairment (Global Deficit Score [GDS] of ≥ 0.5) were invited to participate in this study. Inclusion and exclusion criteria of the RCT have been published elsewhere.[45] We also invited participants who were screened for the RCT but were excluded based on cognitive impairment criteria, to join this study. We included participants established on EFV-based ART for at least 6 months and had suppressed plasma HIV-1 RNA. All participants provided written informed consent. The study was approved by the University of Cape Town Human Research Ethics Committee.

Pharmacokinetic sampling: We collected paired plasma and CSF samples for EFV and metabolites, TFV and FTC assays. Participants recorded dosing time the night before, and were admitted in the morning for pharmacokinetic sampling. Mid-dosing lumbar punctures were performed to collect the CSF. Whole blood samples were collected within 45 minutes of CSF sampling, were centrifuged within 1 hour of collection, aliquoted and stored at -80°C until analysis. CSF samples were aliquoted and stored at -80°C until analysis.

EFV and metabolites, TFV and FTC measurement: Drug assays were performed at 2 laboratories. The analytical laboratory in the Division of Clinical Pharmacology at the University of Cape Town measured total EFV, TFV and FTC concentrations in plasma and CSF using validated liquid chromatography tandem mass

spectrometry (LC/MS-MS) assays. The laboratory subscribes to the National Institute of Allergies and Infectious Diseases Division of AIDS Clinical Pharmacology Quality Assurance Antiretroviral Proficiency Testing Program. Lower limit of quantification (LLOQ) for plasma EFV, TFV and FTC were 19.5 ng/ml, 10.0 ng/ml and 37.5 ng/ml, respectively. For CSF measurements, the LLOQ for total EFV, TFV and FTC were 0.5 ng/ml. The Bioanalytical Facility, Department of Molecular and Clinical Pharmacology at the University of Liverpool measured total CSF 8-OH-EFV, plasma 8-OH-EFV and 7-OH-EFV concentrations in plasma and CSF samples using validated LC/MS-MS assays.[25] We were not able to measure CSF 7-OH-EFV concentrations. The LLOQ for CSF 8-OH-EFV, plasma 8-OH-EFV and plasma 7-OH-EFV was 3.125 ng/ml, 5.0 ng/ml and 5.0 ng/ml, respectively. Concentrations below the limit of quantification (BLQ) were analyzed as missing data.

Characterization of genetic polymorphisms: We extracted human DNA from buffy coats using Qiasymphony®. Genotyping was done using Illumina MEGAEX (Illumina, San Diego, CA). Single nucleotide polymorphisms (SNPs) that were not genotyped were imputed. SNPs were extracted for the following seven genes ± 50KB in each direction: *ABCB1* (301 SNPs)*, ABCC4* (630 SNPs)*, ABCC5* (225 SNPs)*, ABCG2* (164 SNPs)*, CYP2A6/B6* (202 SNPs)*, SLCO1A2* (406 SNPs) and *SLCO2B1* (118 SNPs). SNPs were excluded for genotyping efficiency less than 99%, a 5% minor allele frequency cut-off, and Hardy-Weinberg equilibrium (HWE) *P*-values less than 0.00001. We further performed targeted genotyping of *CYP2B6* 516G→T (rs3745274) and *CYP2A6* -48A→C (rs28399433) by TaqMan™ (Applied Biosystems, Foster City, California, USA), *CYP2B6* 983T→C (rs28399499) and 15582C→T (rs4803419) and *SLCO1B1* 521T→C (rs4149056) and *SLCO1B1* (rs4149032) by MassARRAY® iPLEX Gold (Sequenom Inc., San Diego, California, USA). We confirmed genotypes by visual inspection of plots and all samples were genotyped in duplicate. The final data set included 2049 SNPs from 47 participants. All genotyping was done at the Vanderbilt Technologies for Advanced Genomics (VANTAGE). Laboratory personnel with no knowledge of clinical data performed the genotyping. Metabolizer genotype groups for *CYP2B6* were assigned as follows: extensive metabolizer (*CYP2B6* 15882CC-516GG-983TT or *CYP2B6* 15882CT-516GG-983TT), intermediate metabolizer (*CYP2B6* 15882TT-516GG-983TT or *CYP2B6* 15882CC-516GT-983TT or *CYP2B6* 15882CC-516GG-983CT or *CYP2B6* 15882CT-516GT-983TT or *CYP2B6* 15882CT-516GG-983CT) and slow metabolizer (*CYP2B6* 15882CC-516TT-983TT or *CYP2B6* 15882CC-516GT-

983CT or *CYP2B6* 15882CC-516GG-983CC). Furthermore, among participants with slow metabolizer genotype, additional assessment of *CYP2A6* -48A→C (rs28399433) was assessed to categorize the metabolizer status into an ordinal 12-level metabolizer status as described elsewhere. [10,46]

Neurocognitive performance: We assessed neurocognitive impairment by summarizing the neuropsychological test results of selected cognitive domains, adjusting for age, education, gender and ethnicity using appropriate norms, to provide a GDS.[47] We previously reported the domains and tests included.[45] Neurocognitive impairment was defined as a GDS ≥ 0.5. We screened for symptoms of depression using the Center for Epidemiologic Studies Depression (CES-D) scale.[48]

EFV and metabolite neurotoxicity: We compared CSF EFV and 8-OH-EFV concentrations with concentrations reported to be associated with neuronal damage *in vitro* (31.6 ng/ml and 3.3 ng/ml respectively).[23]

Viral load assessment: We determined HIV-1 RNA concentrations in plasma and CSF using the Abbott RealTi*m*e HIV-1 assay (Abbott Park, Illinois, U.S.A.). We considered participants to be virologically suppressed if the viral load was < 400 copies/ml. In plasma and CSF, the lower limit of detection was 40 copies per ml. In CSF, we performed a previously described nested polymerase chain reaction (PCR) and automated DNA sequencing method to detect HIV-1 transactivator viral protein Tat mutations, which have been associated with HIV-1 associated neurocognitive impairment.[49,50]

Blood-brain-barrier integrity: We calculated the CSF-to-blood albumin ratio (CSF albumin [mg/l]/serum albumin [g/l)] to determine blood-brain-barrier (BBB) integrity. The blood-brain-barrier was considered intact if this ratio was less than 6.8 in participants younger than 45 years of age, and less than 10.2 in participants older than 45 years of age.[51]

Pharmacokinetic statistical analysis: We assessed the normality of data visually and using the Shapiro-Wilk test. Normally-distributed data were described using the means and standard deviations while median and interquartile ranges (IQR) were used for skewed data. Pharmacokinetic data were not normally

distributed and were additionally expressed as geometric means (95% confidence interval). We corrected for plasma protein-binding and estimated protein-free plasma concentrations in our pharmacokinetic analysis by multiplying total plasma concentrations by the protein-free concentrations reported in the literature (EFV 0.22%, TFV 1% and FTC 4%).[52–54] Total CSF concentrations were considered to be similar to CSF protein-free concentrations.[52] Pearson's *r* correlation was used to assess correlations between plasma and CSF concentrations. We performed statistical analysis using STATA version 15.0 (StataCorp, College Station, Texas, USA). Graphs were created using GraphPad Prism version 7.03 for Windows (GraphPad Software, La Jolla, California, USA).

Genetic association analysis: Genetic associations with pharmacokinetic parameters were analyzed by univariate and multivariate linear or logistic regression. Pharmacokinetic data were log-transformed (log_{10}) for genetic analysis. We used ratios of measured concentrations (total in CSF and plasma) without correcting for protein binding. CSF-to-plasma ratios were calculated using raw concentrations then log_{10}-transformed. For EFV analyses, we subsequently adjusted for *CYP2B6* 516G→T, 983T→C and 15582C→T. We performed genetic associations analyses in PLINK version 1.9 (http://zzz.bwh.harvard.edu/plink/). For the primary analyses, we employed LD pruning with a LD r^2 threshold of 0.95 within a 50kb window at 5kb increments. The final analysis dataset included 880 SNPs which met the LD pruning threshold. We used Bonferroni correction to adjust for multiple testing (*P*-value = 0.05 divided by 880 SNPs). We generated a LD plot using Haploview (https://www.broadinstitute.org/haploview/haploview).

Results

Study participant characteristics. We sampled 47 participants (**Table 1**), 33 (70%) of whom had mild to moderate neurocognitive impairment. All participants self-identified as Black Xhosa, and all were virologically suppressed in plasma. Four participants had detectable viral loads, with the highest being 128 copies per ml. CSF viral loads were < 40 copies per ml in all participants. Five participants had detectable HIV-1 Tat DNA, all of whom had the C30C31S substitution. We were able to determine the CSF-to-blood

albumin ratio in 31 (66%) of 47 of participants, with a median value of 2.6 (range 1.1 to 5.2), indicating an intact BBB.

EFV, TFV and FTC pharmacokinetics. Concentrations of EFV (plasma and CSF), 8-OH-EFV (plasma and CSF), 7-OH-EFV (plasma), TFV (plasma and CSF) and FTC (plasma and CSF) are described in **Table 2**. Plasma 8-OH-EFV and 7-OH-EFV concentrations correlated with plasma EFV concentrations ($p < 0.0001$ for each) (**Figure 1, Panels A and B**). Concentrations of EFV, FTC and TFV in CSF correlated with concentrations of these drugs in plasma ($P < 0.0001$ for each) (**Figure 1, Panels C, D and E**). There was no correlation between CSF 8-OH-EFV and plasma EFV, CSF 8-OH-EFV and plasma 8-OH-EFV or plasma 7-OH-EFV and plasma 8-OH-EFV (**Figure 1, Panels F, G and H**). There was no statistically significant association of CSF-to-plasma ratios versus time after dosing, which ranged from approximately 13 hours to 18 hours, for EFV, 8-OH-EFV, TFV or FTC (**Figure 2**). CSF EFV concentrations were above the 50% inhibitory concentration (IC_{50}) 1.3 ng/ml in all participants.[52,55–57] CSF TFV concentrations were below the IC_{50} for TFV (11.5 ng/ml) in all participants and CSF FTC concentrations were below the IC_{50} (70.0 ng/ml) in 21 (49%) of 43 of participants.[54,58] CSF EFV concentrations and CSF 8-OH-EFV concentrations were above the *in vitro* toxic concentration (CSF EFV 31.6 ng/ml; CSF 8-OH-EFV 3.3 ng/ml) in 7 (15.2%) of 46 participants and 14 (29.8%) of 47) participants, respectively.[23]

Genetic associations for EFV

Genotyping of 4 polymorphisms with known effects on EFV (*CYP2B6* 516G→T, 983T→C and 15582C→T and *CYP2A6* -48 A→C) and 2 polymorphisms in *SLCO1B1* (rs4149056 and rs4149032) was successful in all 47 participants. *SLCO1B1* rs4149056 was monomorphic. In 43 (91%) of 47 participants, additional 2043 polymorphisms from *ABCB1, ABCC4, ABCC5, ABCG2, CYP2A6, CYP2B6*, *SLCO1A2* and *SLCO2B1* were successfully genotyped. All 2048 polymorphisms were in HWE based on a Bonferroni adjusted *P*-value threshold of 0.00002; 18 had unadjusted *P*-values <0.05 (data not shown). The 880 polymorphisms included in the final dataset based on LD pruning were in HWE based on a Bonferroni adjusted *P*-value threshold of 5.7×10^{-05}. Ten polymorphisms had unadjusted *P*-values <0.05.

CSF-to-plasma EFV concentration ratios: The linear regression analyses results for associations between genetic polymorphisms and CSF-to-plasma ratios for EFV are displayed in **Table 3**. In univariate linear regression models, of the 880 polymorphisms, rs9584273 in *ABCC4* had the lowest P-value for association with $\log_{10}$-transformed CSF-to-plasma EFV concentration ratio [β=0.14, 95% CI (0.05 to 0.22), P=2.3 x10^{-03}]. This was not statistically significant [Bonferroni corrected P<5.7x10^{-05}]. There was a trend between 11 additional polymorphisms (6 in *ABCC4*, 2 in *ABCC5,* 1 in *ABCB1*, *CYP2B6*, and *CYP2G1P)* and CSF-to-plasma EFV concentration ratios (P<0.01). There were no significant associations between individuals SNPs *CYP2B6* 516G→T, 983T→C, 15582C→T and *CYP2A6* -48A→C and CSF-to-plasma EFV concentration ratios (P>0.05) (**Table 3**). No polymorphisms were significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T, 516G→T 983T→C, 516G→T 983T→C and 15582C→T.

CSF-to-plasma 8-OH-EFV concentration ratios: The linear regression analyses results for associations between genetic polymorphisms and CSF-to-plasma ratios for 8-OH-EFV concentration ratios in 16 participants are displayed in **Table 4**. In univariate linear regression models, of the 880 polymorphisms, *ABCC5* rs6762938 had the lowest P-value for a trend between $\log_{10}$-transformed CSF-to-plasma 8-OH-EFV concentration ratio [β=0.23, 95% CI (0.14 to 0.32), P=2.6 x10^{-04}]. There was a trend between 17 additional polymorphisms (7 in *ABCC4*, 7 in *ABCC5,* 1 in *ABCB1*, *ABCG2* and *SLCO1B1*) and CSF-to-plasma 8-OH-EFV concentration ratios (P <0.01). There were no significant associations between individual SNPs *CYP2B6* 516G→T, 983T→C, 15582C→T and *CYP2A6* -48A→C and CSF-to-plasma 8-OH-EFV concentration ratios (P>0.05) (**Table 4**). No polymorphisms were significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T, 983T→C and 15582C→T.

Plasma EFV concentrations: Relationships between *CYP2B6* slow metabolizer genotypes and EFV concentrations in all individuals are described in **Table 5**. Plasma EFV concentrations were significantly higher in *CYP2B6* slow metabolizers compared with intermediate and extensive metabolizers. **Figure 3** shows concentrations of $\log_{10}$-transformed EFV and metabolites in the plasma and CSF by *CYP2B6/A6* metabolizer status. In univariate linear regression models, composite *CYP2B6* 15582/516/983 genotype

was most strongly associated with plasma EFV concentrations [β=0.34, 95% CI (0.20 to 0.48), P = 1.7 x10^{-05}] (**Supplemental Table S1**). Of the 880 polymorphisms, *CYP2B6* rs60618302 had the lowest *P* value and showed a negative association with $\log_{10}$-transformed plasma EFV concentrations [β=-0.43, 95% CI (-0.62 to 0.25), P = 5.3 x10^{-05}]. Seven additional polymorphisms showed an association with $\log_{10}$-transformed plasma EFV concentrations at P< 0.01 and included *CYP2B6* 516G→T [β=0.30, 95% CI (0.12 to 0.49), P = 2.0 x10^{-03}] and another *CYP2B6* polymorphism (rs59243457) in LD with 516G→T at r2>0.6 [β=0.31, 95% CI (0.12 to 0.49), P = 2.7 x10^{-03}]. There was an association between *CYP2B6* 983T→C and $\log_{10}$-transformed plasma EFV concentrations [β=0.35, 95% CI (0.08 to 0.62), P = 1.4 x10^{-02}]. There was no association with *CYP2B6* 15582C→T [β=-0.23, 95% CI (-0.54 to 0.08), P = 0.15]. To identify independent predictors of EFV concentrations we performed multivariable linear regression analysis adjusted for *CYP2B6* 516G→T. By this analysis, the polymorphism associated at P< 0.05 was *CYP2B6* 983T→C [β=0.38, 95%CI (0.14 to 0.61), P=2.7 x10^{-3})]. In an analysis that adjusted for both *CYP2B6* 516G→T and 983T→C, no additional polymorphism was associated with EFV concentrations at the Bonferroni corrected P value of 5.7 x10^{-5}, the lowest P-value being for *ABCB1* rs115780656 [β=-0.37, 95%CI (-0.58 to -0.17), P=9.7 x10^{-4})]. There was no apparent association with *CYP2B6* 15582C→T [β=-0.00, 95%CI (-0.29 to -0.28), P=0.98)] or *CYP2A6* -48A→C [β=-0.31, 95%CI (-0.62 to 0.00), P=0.05)].

Plasma 8-OH-EFV concentrations: Plasma 8-OH-EFV concentrations were not associated with *CYP2B6* metabolizer status. (**Table 5**). The linear regression analyses for associations between genetic polymorphisms and plasma 8-OH-EFV concentrations are displayed in **Supplemental Table S1**. In univariate linear regression models, of the 880 polymorphisms, *ABCB1* rs115780656 had the lowest P-value for association with $\log_{10}$-transformed plasma 8-OH-EFV concentrations [β=-0.36, 95% CI (-0.55 to -0.18), P=4.3 x10^{-04}]. There was a trend between 11 additional polymorphisms (3 in *ABCG2,* 2 in *ABCB1, 2* in *ABCC4,* 1 in *CYP2G1P*, 1 in *CYP2A6* and 1 in *CYP2A7)* and plasma 8-OH-EFV concentrations (P <0.01). There were no significant associations between individuals SNPs *CYP2B6* 516G→T, 983T→C, 15582C→T or *CYP2A6* -48A→C and plasma 8-OH-EFV concentrations (P>0.05) (**Supplemental Table S1**). No polymorphisms were significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T, or *CYP2B6* 516G→T and 983T→C.

Plasma 7-OH-EFV concentrations: Plasma 7-OH-EFV concentrations were significantly higher in *CYP2B6* slow metabolizers compared with intermediate and extensive metabolizers (**Table 5**). In univariate linear regression models, composite *CYP2B6* genotype best described the association between log_{10}-transformed plasma 7-OH-EFV concentrations [β=0.45, 95% CI (0.10 to 0.66), *P*=5.9 $x10^{-05}$] (**Supplemental Table S1**). Among the individual polymorphisms, *CYP2B6* 516G→T [β=0.39, 95% CI (0.14 to 0.70), *P*=5.5 $x10^{-03}$] and *CYP2B6* 983T→C [β=0.50, 95% CI (0.12 to 0.86), *P*=1.5 $x10^{-02}$] were significantly associated with log_{10}-transformed plasma 7-OH-EFV concentrations. There was a trend between 11 additional polymorphisms (*4* in *CYP2B6,* 4 in *CYP2A6,* 2 in *ABCB1* and 1 in *ABCC4)* and log_{10}-transformed plasma 7-OH-EFV concentrations (*P* <0.01), with the *CYP2A6* rs56164728 having the lowest *P* value [β=-1.02, 95% CI (-1.47 to -0.57), *P*=6.4 $x10^{-05}$]. Furthermore, there were no significant associations between individuals SNPs *CYP2B6* 15582C→T and *CYP2A6* -48A→C and log_{10}-transformed plasma 7-OH-EFV concentrations (*P*>0.3) (**Supplemental Table S1**). After adjusting for *CYP2B6* 516G→T, *CYP2B6* 983T→C remained significant [β=0.59, 95% CI (0.22 to 0.96), *P*=3.7 $x10^{-03}$] and *ABCB1* rs11578656 became significant [β=-0.71, 95% CI (-1.01 to -0.42), *P*=2.9 $x10^{-05}$]. The association between *ABCB1* rs11578656 and log_{10}-transformed plasma 7-OH-EFV concentrations remained significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T and *CYP2B6* 983T→C [β=-0.65, 95% CI (-0.92 to -0.37), *P*=4.1 $x10^{-05}$]. Two additional polymorphisms in *CYP2A6* became significant in multivariate analyses adjusting for *CYP2B6* 516G→T and *CYP2B6* 983T→C; *CYP2A6* rs10853742 [β=-0.55, 95% CI (-0.78 to -0.32), *P*=3.5 $x10^{-05}$] and known polymorphism *CYP2A6* -48A→C [β=-0.59, 95% CI (-1.01 to -0.16), *P*=0.01]. There was no association with between *CYP2B6* 15582C→T and log_{10}-transformed plasma 7-OH-EFV concentrations in univariate (*P*=0.61) or multivariate analyses (*P*>0.60).

CSF EFV concentrations: CSF EFV concentrations were significantly higher in *CYP2B6* slow metabolizers compared with intermediate and extensive metabolizers (**Table 5**). In univariate linear regression models, composite *CYP2B6* genotype best described the association between log_{10}-transformed CSF EFV concentrations [β=0.33, 95% CI (0.17 to 0.48), *P*=1.7 $x10^{-04}$] (**Supplemental Table S2**). Among the individual polymorphisms, *CYP2B6* 516G→T [β=0.29, 95% CI (0.09 to 0.49), *P*=6.2 $x10^{-03}$]

and *CYP2B6* 983T→C [β=0.33, 95% CI (0.04 to 0.61), *P*=3.0 x10^{-02}] were significantly associated with $\log_{10}$-transformed CSF EFV concentrations. There was a trend between 8 additional polymorphisms (3 in *CYP2B6,* 3 in *ABCB1, 1* in *ABCC4* and 1 in *ABBC5)* and $\log_{10}$-transformed CSF EFV concentrations (*P* <0.01). *ABCB1* rs115780656 had the lowest *P*-value for a trend towards a negative association with $\log_{10}$-transformed CSF EFV concentrations [β=-0.51, 95% CI (-0.77 to -0.26), *P*=3.4 x10^{-04}]. There were no significant associations between 15582C→T and *CYP2A6* -48A→C and $\log_{10}$-transformed CSF EFV concentrations (*P*>0.05) (**Supplemental Table S2**). After adjusting for *CYP2B6* 516G→T, the association between *CYP2B6* 983T→C and $\log_{10}$-transformed CSF EFV concentrations remained significant [β=0.36, 95% CI (0.10 to 0.62), *P*=1.0 x10^{-02}]. No polymorphisms were significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T, or *CYP2B6* 516G→T and *CYP2B6* 983T→C.

CSF 8-OH-EFV concentrations*:* There was no relationship between $\log_{10}$-transformed CSF 8-OH-EFV concentrations and *CYP2B6* metabolizer status (**Table 5**). CSF 8-OH-EFV concentrations were only detectable in 16 participants. Linear regression analysis results for associations between polymorphisms and CSF 8-OH-EFV concentrations are displayed in **Supplemental Table S2**. In univariate linear regression models, of the 880 polymorphisms, *ABCC5* rs6762938 had the lowest *P*-value for association with $\log_{10}$-transformed CSF 8-OH-EFV concentrations [β=0.18, 95% CI (0.11 to 0.24), *P*=7.8 x10^{-05}] when the analysis was restricted to the 16 participants with detectable CSF 8-OH-EFV. Ten additional polymorphisms (3 in *ABCC5, 3* in *ABCC4,* 2 in *ABCB1,* 1 in *SLCO2B1* and 1 in *CYP2B6)* showed a trend towards an association with $\log_{10}$-transformed CSF 8-OH-EFV concentrations, including *CYP2B6* 983T→C [β=-0.17, 95% CI (-0.34 to 0.00), *P*=0.08]. There were also no significant associations between composite *CYP2B6* 15582/516/983 genotype or individual polymorphisms *CYP2B6* 516G→T, *CYP2B6* 15582C→T or *CYP2A6* -48A→C and $\log_{10}$-transformed CSF 8-OH-EFV concentrations (*P*>0.05) (**Supplemental Table S2**). *CYP2B6* 983T→C remained not significant after adjusting for *CYP2B6* 516G→T (*P*=0.07). No polymorphisms were significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T, or *CYP2B6* 516G→T and *CYP2B6* 983T→C. In univariate logistic regression analyses in all 47 participants comparing those with detectable CSF 8-OH-EFV concentrations versus those with

undetectable concentrations, there were no significant associations between polymorphisms and detectable CSF 8-hydroxy-efavirenz (**Supplemental Table S2)**.

Plasma 8-OH-EFV/EFV ratios: Regression analysis results for genetic associations with plasma 8-OH-EFV/EFV are displayed in **Supplemental Table S3.** In univariate linear regression models, composite *CYP2B6* 15582/516/983 genotype was most strongly associated with $\log_{10}$-transformed 8-OH-EFV/EFV ratios [β=-0.29, 95% CI (-0.38 to -0.21), *P*=3.7 $x10^{-08}$]. There were also significant associations between $\log_{10}$-transformed 8-OH-EFV/EFV ratio and individual polymorphisms, *CYP2B6* 516G→T [β=-0.27, 95% CI (-0.39 to -0.15), *P*=6.5 $x10^{-05}$] and *CYP2B6* 983T→C [β=-0.28, 95% CI (-0.46 to -0.09), *P*=5.5 $x10^{-03}$]. There were no significant associations between *CYP2B6* 15582C→T or *CYP2A6* -48A→C and $\log_{10}$-transformed 8-OH-EFV/EFV ratios (*P*>0.05) (**Supplemental Table S3**). There were 19 additional polymorphisms (7 in *ABCC4,* 5 in *SLCO2B1,* 4 in *CYP2B6* and 1 in *ABCG2, EGLN2, PKD2* and *CYP2B6* respectively*)* that showed a trend with $\log_{10}$-transformed 8-OH-EFV/EFV ratios (P<0.01). After adjusting for *CYP2B6* 516G→T, the association between *CYP2B6* 983T→C and $\log_{10}$-transformed 8-OH-EFV/EFV ratios remained significant [β=-0.31, 95% CI (-0.45 to -0.16), *P*=1.8 $x10^{-04}$]. No polymorphisms were significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T and *CYP2B6* 983T→C.

Plasma 7-OH-EFV/EFV ratios: In univariate linear regression models, a previously described *CYP2B6* polymorphism (rs2279345) was significantly associated with lower 7-OH-EFV/EFV ratios [β=-0.28, 95% CI (-0.42 to -0.15), *P*=1.8 $x10^{-04}$] at *P*<0.05 (**Supplemental Table S3**). Of the 880 polymorphisms, rs56164728 in *CYP2A6* had the lowest P-value for a trend between $\log_{10}$-transformed 7-OH-EFV/EFV ratios [β=-0.50, 95% CI (-0.72 to -0.27), *P*=1.1 $x10^{-04}$]. Seven additional polymorphisms (3 in *CYP2A6,* 3 in *ABBC4* and 1 in *ABCB1*) showed a trend at *P*<0.01. There were no significant associations between composite *CYP2B6* 15582/516/983 genotype or individual polymorphisms, *CYP2B6* 516G→T, *CYP2B6* 983T→C, *CYP2B6* 15582C→T or *CYP2A6* -48A→C and $\log_{10}$-transformed plasma 7-OH-EFV/EFV ratios (*P*>0.05) (**Supplemental Table S3**). After adjusting for *CYP2B6* 516G→T, *CYP2B6* 516G→T and *CYP2B6*

983T→C, the association between *CYP2B6* polymorphism (rs2279345) and lower 7-OH-EFV/EFV ratios remained significant at *P*<0.05.

CSF 8-OH-EFV/EFV ratios: Linear regression analysis results for genetic associations with log_{10}-transformed CSF 8-OH-EFV/EFV ratios were available only in 16 participants and are displayed in **Supplemental Table S3**. In univariate linear regression models, 2 polymorphisms in *ABCC5* showed a trend towards an association with higher log_{10}-transformed CSF 8-OH-EFV/EFV ratios at *P*<0.01. In addition, rs76268776 downstream from *CYP2A6* (*P*<0.01) and the composite *CYP2B6* 518/983 genotype (*P*<0.1) showed trends towards an association with lower CSF 8-OH-EFV/EFV ratios. After adjusting for *CYP2B6* 516G→T and *CYP2B6* 983T→C, the association between *CYP2A6* -48A→C and higher log_{10}-transformed CSF 8-OH-EFV/EFV ratios became significant [β=0.55, 95% CI (0.05 to 1.03), *P*=4.8 $x10^{-02}$].

Genetic polymorphisms and TFV concentrations

Plasma TFV concentrations: In univariate linear regression models, and correcting for multiple testing, there were no significant associations between any of the 880 polymorphisms and plasma TFV concentrations (**Supplemental Table S4**). Ten polymorphisms (3 in *ABCG2*, 3 in *ABCC5*, 3 in *SLCO1A2* and 1 in *ABCC4*) were associated with lower plasma TFV concentrations at *P*<0.01, with *ABCG2* (rs2231159) having the lowest *P* value [β=-0.32, 95% CI (-0.48 to -0.17), *P*=2.2$x10^{-04}$].

CSF TFV concentrations: In univariate linear regression models, and correcting for multiple testing, there were no significant associations between any of the 880 polymorphisms CSF concentrations (**Supplemental Table S4**). Six polymorphisms (2 in *ABCB1*, 1 in *ABCG2,* 1 in *ABCC5*, 1 in *SLCO1A2* and 1 in *ABCC4*) were associated with lower CSF TFV concentrations at *P*<0.01, with *ABCC4* (rs7982526) having the lowest *P* value [β=-0.58, 95% CI (-0.85 to -0.30), *P*=1.7 $x10^{-04}$]. In addition, *SLCO1A2* (rs140377659) was associated with higher CSF TFV concentrations at *P*<0.01.

CSF-to-plasma TFV ratios: In univariate linear regression models, and correcting for multiple testing, there were no significant associations between any of the 880 polymorphisms and CSF-to-plasma TFV ratios (**Supplemental Table S4**). Eleven polymorphisms (3 in *ABCB1,* 2 in *ABCG2*, 6 *SLCO1A2*) were

associated with CSF-to-plasma TFV ratios at *P*<0.01, with *ABCG2* (rs1989830) having the lowest *P* value [β=-0.12, 95% CI (-0.19 to -0.05), $P=2.2x10^{-04}$]. Polymorphisms in *SLCO1A2* showed a trend towards higher ratios, whereas polymorphisms in *ABCB1 and ABCG2* showed trends with lower ratios.

Genetic polymorphisms associated with FTC concentrations

Plasma FTC concentrations: Only 39 participants were included in the analyses. In univariate linear regression models, and correcting for multiple testing, there were no significant associations between any of the 880 polymorphisms and plasma FTC concentrations (**Supplemental Table S5**). Six polymorphisms (3 in *ABCC5*, 1 in *SLCO1A2* and 1 in *ABCC4* and 1 in *SLCO2B1*) were associated with plasma FTC concentrations at *P*<0.01, with *ABCG5* rs56889675 having the lowest *P* value [β=-0.26, 95% CI (-0.40 to -0.12), $P=8.0\ x10^{-04}$]. *ABCC5* rs74763842 showed a trend towards an association with higher plasma FTC, while the remaining polymorphisms towards an association with lower FTC concentrations.

CSF FTC concentrations: In univariate linear regression models, and correcting for multiple testing, there were no significant associations between any of the 880 polymorphisms CSF FTV concentrations (**Supplemental Table S5**). Twelve polymorphisms (2 in *ABCB1*, 1 in *ABCG2,* 1 in *ABCC5*, 1 in *SLCO1A2* and 1 in *ABCC4*) were associated with lower CSF FTC concentrations at *P*<0.01, with *ABCC5* rs56889675 having the lowest *P* value [β=-0.32, 95% CI (-0.50 to -0.15), $P=7.2\ x10^{-04}$].

CSF-to-plasma FTC ratios: In univariate linear regression models, and correcting for multiple testing, there were no significant associations between any of the 880 polymorphisms and CSF-to-plasma FTC ratios (**Supplemental Table S5**). Eight polymorphisms (3 in *ABCC5,* 3 in *ABCC4,* 1 in *ABCG2*, 1 in *SLCO1A2*) were associated with CSF-to-plasma FTV ratios at *P*<0.01, with *ABCC5* rs11921035 having the lowest *P* value [β=-0.32, 95% CI (-0.50 to -0.14), $P=1.4\ x10^{-03}$]. Two polymorphisms in *ABCC4* showed a trend towards higher ratios, whereas the remaining polymorphisms showed trends with lower ratios.

Pharmacokinetic-pharmacodynamic associations with neurocognitive performance

We found no significant correlation between GDS and CSF concentrations of EFV, 8-OH-EFV, TFV or FTC. Detectable CSF 8-OH-EFV concentration tended to be associated with impaired executive function on the Colour Trails Test (P = 0.043). Participants with detectable CSF 8-OH-EFV had a higher GDS compared to participants without detectable CSF 8-OH-EFV (1.0 compared to 0.82), but this was not statistically significant. The GDS in the 5 participants in whom HIV-1 Tat DNA were detected, were similar to those in whom Tat DNA were not detected.

Discussion

We investigated whether genetic polymorphisms are associated with CSF disposition of EFV, TFV and FTC in Black South African adults. A model that included composite *CYP2B6* 15582/516/983 genotype in univariate analyses best predicted $\log_{10}$-transformed concentrations of plasma EFV, plasma 7-OH-EFV, plasma 8-OH-EFV/EFV ratio and CSF EFV. The individual polymorphisms *CYP2B6* 516G→T and 983T→C also predicted these concentrations. Lower plasma 7-OH-EFV concentrations were independently associated with *CYP2A6* rs10853742, *ABCB1* rs115780656 and *CYP2A6* -48A→C. The *CYP2A6* -48A→C polymorphism was also independently associated with higher CSF 8-OH-EFV/EFV ratio. The *CYP2B6* rs2279345 polymorphism was associated with lower plasma 7-OH-EFV/EFV ratio in univariate and multivariate analyses adjusting for *CYP2B6* 516G→T and 983T→C (P<0.05). No polymorphisms were associated with CSF-to-plasma ratios of all 3 drugs, plasma or CSF 8-OH-EFV, TDF or FTC concentrations or neurocognitive performance.

We expected CSF 8-OH-EFV concentrations to be higher in extensive metabolizers as it is formed via the *CYP2B6* enzymatic pathway. However, similar to the findings of others, 8-OH-EFV concentrations in plasma and CSF remained constant irrespective of metabolizer status. [22,25,26] It is possible that the small number of participants (16 of 47) with detectable CSF 8-OH-EFV concentrations contributed to the lack of the association between the metabolizer status and CSF 8-OH-EFV concentrations. Winston and colleagues proposed that CSF 8-OH-EFV concentrations are independent of metabolizer status as it is 8-OH-EFV spill over from the plasma, or that EFV is metabolized to 8-OH-EFV in the CNS, trapping 8-OH-

EFV within the CNS compartment.[22] We found that *CYP2A6* -48A→C was independently associated with higher CSF 8-OH-EFV/EFV ratio, which may suggest that in *CYP2B6* slow metabolizers, EFV may be metabolized to 8-OH-EFV in the CNS by the accessory pathway *CYP2A6*. CSF 8-OH-EFV further undergoes phase II glucuronidation and sulfation; both CSF 8-OH-EFV-glucuronide and CSF 8-OH-EFV-sulfate concentrations exceed by several fold that of the parent CSF 8-OH-EFV.[26] Phase II metabolism enhances the polarity of CSF 8-OH-EFV into a less lipid-soluble molecule.[26] However we did not find a statistically significant association with *CYP2A6* -48A→C and CSF 8-OH-EFV or plasma 8-OH-EFV.

We explored pharmacokinetic-pharmacodynamic relationships of EFV-TFV-FTC and neurocognition. Although CSF EFV and 8-OH-EFV concentrations were above the *in vitro* CSF toxicity threshold, in 15.2% and 29.8% of participants respectively, we did not find a relationship between GDS performance and plasma or CSF EFV-TFV-FTC concentrations or CSF 8-OH-EFV concentrations. Participants with detectable CSF 8-OH-EFV scored worse on the Colour Trails Test (*P*-value = 0.04) and had a higher GDS (median 1.39 compared to median 1.0), but this was not statistically significant. It is possible that *CYP2A6* -48A→C predisposes *CYP2B6* slow metabolizers to higher CSF 8-OH-EFV concentrations and worse neurocognitive performance. Various cellular mechanisms for EFV toxicity have been proposed.[59] Higher EFV concentrations, which are associated with *CYP2B6* slow metabolizer status, are associated with neurological symptoms, which may include serious presentations such as encephalopathy.[21,60] In a CSF sub-study of the ENCORE1 trial, 8-OH-EFV exposure correlated with adverse neuropsychology outcomes as measured by the Depression Anxiety Stress Scale and EFV Symptom Questionnaire at 4 and 48 weeks of EFV initiation.[22] CSF concentrations of EFV, 8-OH-EFV and the phase II metabolites of 8-OH-EFV have not been associated with EFV treatment discontinuations.[26] Previous animal studies showed an association between the C30C31S substitution and a possible neuroprotective effect. However studies in HIV-1 infected humans, including ours, do not confirm this association. The neurocognition scores (GDS) of the 5 participants with C30C31S substitution were similar to other participants'.[50,61]

Our study has limitations. We only studied 47 participants. We had limited power to detect genetic associations between infrequent genotypes with small effect sizes (increase in plasma or CSF

concentrations). For example, the *CYP2A6* -48A→C polymorphism has been associated with increased plasma EFV concentrations in *CYP2B6* slow metabolizers,[46] but we found no association as there only 3 participants with *CYP2B6* slow metabolizer genotype that carried a single *CYP2A6* -48A→C allele. This may have also limited our ability to detect associations between *CYP2B6* 15582C→T and plasma EFV concentrations, as 15582CT heterozygosity has been associated with small increases in plasma EFV exposure, and there were no 15582TT homozygotes in our study.[10] We were also not able to detect pharmacokinetic-pharmacodynamic associations due to limited power to detect smaller differences in cognitive impairment. However, to our knowledge this is the largest sample size examining pharmacogenetic, pharmacokinetic and pharmacodynamic associations with CSF EFV-TFV-FTC. Our study was cross-sectional. Neurocognitive changes would have been better assessed longitudinally. We did not measure unbound concentrations of EFV-TFV-FTC. EFV is 99.8% protein bound, but protein-free EFV concentrations are equivalent in blood plasma and CSF.[52] TFV and FTC are less than 7% and 4% protein bound, respectively.[54] We assessed CSF-to-plasma ratios of total concentrations. Protein-free CSF-to-plasma concentrations of EFV in particular may have more accurately reflected the pharmacodynamically active concentrations. We did not measure the phase II EFV metabolites in CSF, which exceed concentrations of CSF EFV and CSF 8-OH-EFV.[26] The effect of phase II EFV metabolites on neurocognition are unknown. As our study only included adults, pharmacokinetic and pharmacodynamic findings may not apply to a paediatric population. There are concerns about the limited safety data regarding EFV and neurocognitive development of children.[62] Our inability to demonstrate a neurocognitive association with EFV-TFV-FTC pharmacokinetics in adults cannot be extrapolated to children.

In summary, we investigated polymorphisms associated with CSF exposure of EFV, 8-OH-EFV, TFV and FTC in black South Africans. We also explored the pharmacokinetic-pharmacodynamic relationships of CSF EFV-TFV-FTC with neurocognitive performance. To our knowledge this is the largest study to examine pharmacogenetic, pharmacokinetic and pharmacodynamic associations with CSF EFV-TFV-FTC. We identified novel genetic associations with plasma EFV, plasma 7-OH-EFV, plasma 7-OH-EFV/EFV ratio, plasma 8-OH-EFV/EFV ratio and CSF efavirenz. No polymorphisms were associated with CSF-to-

plasma ratios of EFV, TFV or FTC; plasma or CSF 8-OH-EFV, TFV or FTC concentrations; or neurocognitive performance.

Acknowledgements

The study participants.

The study team in alphabetical order: Laura Comrie (clinical support), Carla Freeman (clinical support), Shahieda Isaacs (viral sequencing), Pam Jordan (data capturer), Teboho Linda (neuropsychology technician). Nozipho Mawisa (study nurse), Queen Maswana (recruiter), Rasmita Ori (clinical support), Kareema Poggenpoel (administration support) and Shireen Surtie (study coordinator). We are grateful to Cara Sutcliffe and the VANTAGE team at Vanderbilt.

Funding

This work was supported by the South African Medical Research Council and the European and Developing Countries Clinical Trials Partnership (SP.2011.41304.065). The drug assays analysed at UCT were supported in part by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (UM1 AI068634, UM1 AI068636, UM1 AI106701, U01 AI068632), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (AI068632). DWH is supported by National Institutes of Health grants AI077505, AI069439 and TR002243.

Transparency declarations

The authors report no conflicts of interest.

References

1. World Health Organisation (WHO). First-line ART for Adults. http://www.who.int/hiv/pub/guidelines/arv2013/art/artadults/en/. Accessed June 1, 2017.
2. Underwood J, Robertson KR, Winston A. Could antiretroviral neurotoxicity play a role in the pathogenesis of cognitive impairment in treated HIV disease?. *AIDS*. 2015;29(3):253-261. doi:10.1097/QAD.0000000000000538.
3. Robertson K, Liner J, Meeker RB. Antiretroviral neurotoxicity. *J Neurovirol*. 2012;18(5):388-399. doi:10.1007/s13365-012-0120-3.
4. Robertson KR, Su Z, Margolis DM, et al. Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology*. 2010;74(16):1260-1266. doi:10.1212/WNL.0b013e3181d9ed09.
5. Winston A, Duncombe C, Li PCK, et al. Does choice of combination antiretroviral therapy (cART) alter changes in cerebral function testing after 48 weeks in treatment-naive, HIV-1-infected individuals commencing cART? A randomized, controlled study. *Clin Infect Dis*. 2010;50(6):920-929. doi:10.1086/650743.
6. Letendre S, Marquie-Beck J, Capparelli E, et al. Validation of the CNS penetration-effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Arch Neurol*. 2008;65(1):65-70. doi:10.1001/archneurol.2007.31.
7. Ellis RJ, Letendre S, Vaida F, et al. Randomized trial of central nervous system-targeted antiretrovirals for HIV-associated neurocognitive disorder. *Clin Infect Dis*. 2014;58(7):1015-1022. doi:10.1093/cid/cit921.
8. Caniglia EC, Cain LE, Justice A, et al. Antiretroviral penetration into the CNS and incidence of AIDS-defining neurologic conditions. *Neurology*. 2014;83(2):134-141. doi:10.1212/WNL.0000000000000564.
9. Haas DW, Tarr PE. Perspectives on pharmacogenomics of antiretroviral medications and HIV-associated comorbidities. *Curr Opin HIV AIDS*. 2015;10(2):116-122. doi:10.1097/COH.0000000000000134.
10. Holzinger ER, Grady B, Ritchie MD, et al. Genome-wide association study of plasma efavirenz

pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. *Pharmacogenet Genomics*. 2012;22(12):858-867. doi:10.1097/FPC.0b013e32835a450b.

11. Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *Aids*. 2004;18(18):2391-2400. doi:00002030-200412030-00006 [pii].
12. Viljoen M, Karlsson MO, Meyers TM, Gous H, Dandara C, Rheeders M. Influence of CYP2B6 516G>T polymorphism and interoccasion variability (IOV) on the population pharmacokinetics of efavirenz in HIV-infected South African children. *Eur J Clin Pharmacol*. 2012;68(4):339-347. doi:10.1007/s00228-011-1148-7.
13. Ngaimisi E, Habtewold A, Minzi O, et al. Importance of ethnicity, CYP2B6 and ABCB1 genotype for efavirenz pharmacokinetics and treatment outcomes: a parallel-group prospective cohort study in two sub-Saharan Africa populations. Emery S, ed. *PLoS One*. 2013;8(7):e67946. doi:10.1371/journal.pone.0067946.
14. Jamshidi Y, Moreton M, McKeown DA, et al. Tribal ethnicity and CYP2B6 genetics in Ugandan and Zimbabwean populations in the UK: implications for efavirenz dosing in HIV infection. *J Antimicrob Chemother*. 2010;65(12):2614-2619. doi:10.1093/jac/dkq369.
15. Kwara A, Lartey M, Sagoe KWCK, Kenu E, Court MH. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. *AIDS*. 2009;23(16):2101-2106. doi:10.1097/QAD.0b013e3283319908.CYP2B6.
16. Swart M, Skelton M, Ren Y, Smith P, Takuva S, Dandara C. High predictive value of CYP2B6 SNPs for steady-state plasma efavirenz levels in South African HIV/AIDS patients. *Pharmacogenet Genomics*. 2013;23(8):415-427. doi:10.1097/FPC.0b013e328363176f.
17. Gounden V, van Niekerk C, Snyman T, George JA. Presence of the CYP2B6 516G> T polymorphism, increased plasma Efavirenz concentrations and early neuropsychiatric side effects in South African HIV-infected patients. *AIDS Res Ther*. 2010;7:32. doi:10.1186/1742-6405-7-32.
18. Sinxadi PZ, Leger PD, McIlleron HM, et al. Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa. *Br J Clin Pharmacol*. 2015;80(1):146-156. doi:10.1111/bcp.12590.

19. Thorn CF, Lamba JK, Lamba V, Klein TE, Altman RB. PharmGKB summary: very important pharmacogene information for CYP2B6. *Pharmacogenet Genomics*. 2010;20(8):520-523. http://www.ncbi.nlm.nih.gov/pubmed/20648701.

20. di Iulio J, Fayet A, Arab-Alameddine M, et al. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. *Pharmacogenet Genomics*. 2009;19(4):300-309. doi:10.1097/FPC.0b013e328328d577.

21. Variava E, Sigauke FR, Norman J, et al. Brief Report: Late Efavirenz-Induced Ataxia and Encephalopathy: A Case Series. *J Acquir Immune Defic Syndr*. 2017;75(5):577-579. doi:10.1097/QAI.0000000000001451.

22. Winston A, Amin J, Clarke A, et al. Cerebrospinal fluid exposure of efavirenz and its major metabolites when dosed at 400 mg and 600 mg once daily: a randomized controlled trial. *Clin Infect Dis*. 2015;60(7):1026-1032. doi:10.1093/cid/ciu976.

23. Tovar-y-Romo LB, Bumpus NN, Pomerantz D, et al. Dendritic spine injury induced by the 8-hydroxy metabolite of efavirenz. *J Pharmacol Exp Ther*. 2012;343(3):696-703. doi:10.1124/jpet.112.195701.

24. Brandmann M, Nehls U, Dringen R. 8-Hydroxy-efavirenz, the primary metabolite of the antiretroviral drug Efavirenz, stimulates the glycolytic flux in cultured rat astrocytes. *Neurochem Res*. 2013;38(12):2524-2534. doi:10.1007/s11064-013-1165-2.

25. Nightingale S, Chau TTH, Fisher M, et al. Efavirenz and Metabolites in Cerebrospinal Fluid: Relationship with CYP2B6 c.516G→T Genotype and Perturbed Blood-Brain Barrier Due to Tuberculous Meningitis. *Antimicrob Agents Chemother*. 2016;60(8):4511-4518. doi:10.1128/AAC.00280-16.

26. Aouri M, Barcelo C, Ternon B, et al. In vivo profiling and distribution of known and novel phase i and phase II metabolites of efavirenz in plasma, urine, and cerebrospinal fluid. *Drug Metab Dispos*. 2016;44(1):151-161. doi:10.1124/dmd.115.065839.

27. Suhy AM, Webb A, Papp AC, Geier EG, Sadee W. Expression and splicing of ABC and SLC transporters in the human blood-brain barrier measured with RNAseq. *Eur J Pharm Sci*. 2017;103:47-51. doi:10.1016/j.ejps.2017.02.010.

28. Varatharajan L, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary

of current knowledge and recommendations for further research. *Antiviral Res*. 2009;82(2):A99-109. doi:10.1016/j.antiviral.2008.12.013.

29. Alfirevic A, Durocher J, Elati A, et al. Misoprostol-induced fever and genetic polymorphisms in drug transporters SLCO1B1 and ABCC4 in women of Latin American and European ancestry. *Pharmacogenomics*. 2015;16(9):919-928. doi:10.2217/pgs.15.53.
30. Stieger B, Gao B. Drug transporters in the central nervous system. *Clin Pharmacokinet*. 2015;54(3):225-242. doi:10.1007/s40262-015-0241-y.
31. Eyal S, Hsiao P, Unadkat JD. Drug interactions at the blood-brain barrier: fact or fantasy? *Pharmacol Ther*. 2009;123(1):80-104. doi:10.1016/j.pharmthera.2009.03.017.
32. Störmer E, von Moltke LL, Perloff MD, Greenblatt DJ. Differential modulation of P-glycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. *Pharm Res*. 2002;19(7):1038-1045. http://www.ncbi.nlm.nih.gov/pubmed/12180537.
33. Swart M, Ren Y, Smith P, Dandara C. ABCB1 4036A>G and 1236C>T Polymorphisms Affect Plasma Efavirenz Levels in South African HIV/AIDS Patients. *Front Genet*. 2012;3(NOV):1-10. doi:10.3389/fgene.2012.00236.
34. Sánchez Martín A, Cabrera Figueroa S, Cruz Guerrero R, Hurtado LP, Hurlé AD-G, Carracedo Álvarez A. Impact of pharmacogenetics on CNS side effects related to efavirenz. *Pharmacogenomics*. 2013;14(10):1167-1178. doi:10.2217/pgs.13.111.
35. Sánchez-Martín A, Cabrera Figueroa S, Cruz R, et al. Gene-gene interactions between DRD3, MRP4 and CYP2B6 polymorphisms and its influence on the pharmacokinetic parameters of efavirenz in HIV infected patients. *Drug Metab Pharmacokinet*. 2016;31(5):349-355. doi:10.1016/j.dmpk.2016.06.001.
36. Reid G, Wielinga P, Zelcer N, et al. Characterization of the transport of nucleoside analog drugs by the human multidrug resistance proteins MRP4 and MRP5. *Mol Pharmacol*. 2003;63(5):1094-1103. http://www.ncbi.nlm.nih.gov/pubmed/12695538.
37. Reznicek J, Ceckova M, Cerveny L, Müller F, Staud F. Emtricitabine is a substrate of MATE1 but not of OCT1, OCT2, P-gp, BCRP or MRP2 transporters. *Xenobiotica*. 2017;47(1):77-85. doi:10.3109/00498254.2016.1158886.

38. Neumanova Z, Cerveny L, Ceckova M, Staud F. Interactions of tenofovir and tenofovir disoproxil fumarate with drug efflux transporters ABCB1, ABCG2, and ABCC2; role in transport across the placenta. *AIDS*. 2014;28(1):9-17. doi:10.1097/QAD.0000000000000112.

39. Imaoka T, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. *Mol Pharmacol*. 2007;71(2):619-627. doi:10.1124/mol.106.028233.

40. Best BM, Letendre SL, Koopmans P, et al. Low cerebrospinal fluid concentrations of the nucleotide HIV reverse transcriptase inhibitor, tenofovir. *J Acquir Immune Defic Syndr*. 2012;59(4):376-381. doi:10.1097/QAI.0b013e318247ec54.

41. Baxi SM, Greenblatt RM, Bacchetti P, et al. Evaluating the association of single-nucleotide polymorphisms with tenofovir exposure in a diverse prospective cohort of women living with HIV. *Pharmacogenomics J*. May 2017. doi:10.1038/tpj.2017.3.

42. Kis O, Robillard K, Chan GNY, Bendayan R. The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol Sci*. 2010;31(1):22-35. doi:10.1016/j.tips.2009.10.001.

43. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. *Annu Rev Genomics Hum Genet*. 2008;9:403-433. doi:10.1146/annurev.genom.9.081307.164258.

44. World Health Organisation. Antiretroviral therapy coverage data and estimates by country. Global Health Observatory data repository. http://apps.who.int/gho/data/node.main.626?lang=en. Published 2017. Accessed April 23, 2018.

45. Decloedt EH, Freeman C, Howells F, et al. Moderate to severe HIV-associated neurocognitive impairment: A randomized placebo-controlled trial of lithium. *Medicine (Baltimore)*. 2016;95(46):e5401. doi:10.1097/MD.0000000000005401.

46. Haas DW, Kwara A, Richardson DM, et al. Secondary metabolism pathway polymorphisms and plasma efavirenz concentrations in HIV-infected adults with CYP2B6 slow metabolizer genotypes. *J Antimicrob Chemother*. 2014;69(8):2175-2182. doi:10.1093/jac/dku110.

47. Carey CL, Woods SP, Gonzalez R, et al. Predictive Validity of Global Deficit Scores in Detecting

Neuropsychological Impairment in HIV Infection. *J Clin Exp Neuropsychol*. 2004;26(3):307-319. doi:10.1080/13803390490510031.

48. Myer L, Smit J, Roux L Le, Parker S, Stein DJ, Seedat S. Common mental disorders among HIV-infected individuals in South Africa: prevalence, predictors, and validation of brief psychiatric rating scales. *AIDS Patient Care STDS*. 2008;22(2):147-158. doi:10.1089/apc.2007.0102.
49. Mishra M, Vetrivel S, Siddappa NB, Ranga U, Seth P. Clade-specific differences in neurotoxicity of human immunodeficiency virus-1 B and C Tat of human neurons: significance of dicysteine C30C31 motif. *Ann Neurol*. 2008;63(3):366-376. doi:10.1002/ana.21292.
50. Paul RH, Joska JA, Woods C, et al. Impact of the HIV Tat C30C31S dicysteine substitution on neuropsychological function in patients with clade C disease. *J Neurovirol*. 2014;20(6):627-635. doi:10.1007/s13365-014-0293-z.
51. Blennow K, Fredman P, Wallin A, et al. Protein analysis in cerebrospinal fluid. II. Reference values derived from healthy individuals 18-88 years of age. *Eur Neurol*. 1993;33(2):129-133. http://www.ncbi.nlm.nih.gov/pubmed/8467819.
52. Avery LB, Sacktor N, McArthur JC, Hendrix CW. Protein-free efavirenz concentrations in cerebrospinal fluid and blood plasma are equivalent: applying the law of mass action to predict protein-free drug concentration. *Antimicrob Agents Chemother*. 2013;57(3):1409-1414. doi:10.1128/AAC.02329-12.
53. Kearney BP, Flaherty JF, Shah J. Tenofovir disoproxil fumarate: clinical pharmacology and pharmacokinetics. *Clin Pharmacokinet*. 2004;43(9):595-612. doi:10.2165/00003088-200443090-00003.
54. Yilmaz A, Price RW, Gisslén M. Antiretroviral drug treatment of CNS HIV-1 infection. *J Antimicrob Chemother*. 2012;67(2):299-311. doi:10.1093/jac/dkr492.
55. Best BM, Koopmans PP, Letendre SL, et al. Efavirenz concentrations in CSF exceed IC50 for wild-type HIV. *J Antimicrob Chemother*. 2011;66(2):354-357. doi:10.1093/jac/dkq434.
56. Avery LB, VanAusdall JL, Hendrix CW, Bumpus NN. Compartmentalization and antiviral effect of efavirenz metabolites in blood plasma, seminal plasma, and cerebrospinal fluid. *Drug Metab Dispos*. 2013;41(2):422-429. doi:10.1124/dmd.112.049601.

57. Yilmaz A, Watson V, Dickinson L, Back D. Efavirenz pharmacokinetics in cerebrospinal fluid and plasma over a 24-hour dosing interval. *Antimicrob Agents Chemother*. 2012;56(9):4583-4585. doi:10.1128/AAC.06311-11.

58. Best BM, Letendre SL, Koopmans P, et al. Low CSF Concentrations of the Nucleotide HIV Reverse Transcriptase Inhibitor, Tenofovir. *J Acquir Immune Defic Syndr*. 2013;59(4):376-381. doi:10.1097/QAI.0b013e318247ec54.LOW.

59. Apostolova N, Blas-Garcia A, Galindo MJ, Esplugues J V. Efavirenz: What is known about the cellular mechanisms responsible for its adverse effects. *Eur J Pharmacol*. 2017;812(April):163-173. doi:10.1016/j.ejphar.2017.07.016.

60. Kenyon C, Mfolozi S, Croxford R, Colebunders R, Cohen K. Severe efavirenz-induced vacuolar axonopathy complicated by fatal aspiration pneumonia. *Br J Clin Pharmacol*. 2012;74(6):1070-1072. doi:10.1111/j.1365-2125.2012.04299.x.

61. Dara J, Dow A, Cromwell E, et al. Multivariable analysis to determine if HIV-1 Tat dicysteine motif is associated with neurodevelopmental delay in HIV-infected children in Malawi. *Behav Brain Funct*. 2015;11:38. doi:10.1186/s12993-015-0083-7.

62. Van de Wijer L, Schellekens AFA, Burger DM, Homberg JR, de Mast Q, van der Ven AJAM. Rethinking the risk-benefit ratio of efavirenz in HIV-infected children. *Lancet Infect Dis*. 2016;16(5):e76-e81. doi:10.1016/S1473-3099(16)00117-1.

Table 1. Baseline characteristics of study participants (n=47)

Characteristic	
Gender	
Male, n (%)	6 (15%)
Female, n (%)	41 (85%)
Age in years[†]	36 (32 – 43)
CD4+ T-cell count[†] (cells/mm^3)	470 (384 – 586)
Months on ART[†] (months)	38 (18 – 54)
Body mass index[‡] (kg/m^2)	26.3 ± 5.3
ART regimen	
EFV-TFV-FTC, n (%)	43 (91%)
EFV-TFV-3TC, n (%)	4 (9%)
Neurocognitive impairment	
GDS overall[†]	0.89 (0.22 – 1.5)
GDS ≥ 1, n (%)	22 (47%)
GDS > 0.5 < 1, n (%)	11 (23%)
GDS < 0.5, n (%)	14 (30%)
Neuromedical assessment	
No disease, n (%)	26 (55%)
Mild-moderate disease, n (%)	21 (46%)
Severe disease, n (%)	0
Years education	
≥ 10, n (%)	24 (51%)
< 10, n (%)	21 (45%)
Missing information, n (%)	2 (4%)
Employment status	
Employed[§], n (%)	12 (26%)
Unemployed, n (%)	35 (74%)
Depression score	
CES-D[†]	7 (2 – 11)

[†]Median and interquartile range; [‡]Mean and standard deviation [§]Full-time or part-time work; EFV-TFV-FTC = efavirenz-tenofovir-emtricitabine; EFV-TFV-3TC = efavirenz-tenofovir-lamivudine; GDS = Global Deficit Score; CES-D = Center for Epidemiologic Studies Depression scale

Table 2. Concentrations of efavirenz and its metabolites, tenofovir and emtricitabine in plasma and cerebrospinal fluid.

	Plasma (ng/ml): Total concentration	**Plasma (ng/ml): Protein corrected**	**CSF concentration (ng/ml)**	**CSF/plasma ratio: Total concentration (%)**	**CSF/plasma ratio: Protein corrected (%)**
Efavirenz pharmacokinetics (n=47)					
Undetectable samples (%)	0	0	1/47 (2.1%)	1/47 (2.1%)	1/47 (2.1%)
Median (IQR)	1960 (1390 – 3200)	4.31 (3.06 - 7.04)	17.25 (10.7 – 19.9)	0.71 (0.61 - 0.78)	324.34 (278.29 – 356.44)
Range	55 – 18100	0.12 - 39.82	1.73 – 119	0.31 - 1.12	142.82 – 508.66
Geometric mean concentration (95% CI)	2081.5 (1557.8 – 2781.4)	4.58 (3.43 – 6.12)	15.64 (12.08 – 20.24)	0.69 (0.64 – 0.75)	315.54 (291.90 – 341.10)
8-Hydroxy-efavirenz pharmacokinetics (n=47)					
Undetectable samples (%)	0	Unknown	30/47 (63.8%)	30/47 (63.8%)	Unknown
Median (IQR)	1808 (1325.5 – 2498.7)		4.17 (3.80 – 5.79)	0.20 (0.14 – 0.24)	
Range	68.81 – 4887.5		(3.15 – 9.56)	0.10 – 0.72	
Geometric mean concentration (95% CI)	1570.7 (1255 – 1965.9)		4.69 (3.93 – 5.60)	0.21 (0.16 – 0.26)	
7-Hydroxy-efavirenz pharmacokinetics (n=47)					
Undetectable samples (%)	2/47 (4.3%)	Unknown	Not measured	Not applicable	Not applicable
Median (IQR)	216.71 (122.91 – 375.43)				
Range	11.45 – 2181.73				
Geometric mean concentration (95% CI)	229.17 (166.51 – 315.41)				
Tenofovir pharmacokinetics (n=47)					
Undetectable samples (%)	3/47 (6.4%)	3/47 (6.4%)	4/47 (8.5%)	4/47 (8.5%)	4/47 (8.5%)

Median (IQR)	63.45 (50.75 - 81.2)	0.63 (0.51 – 0.81)	1.4 (1.07 - 2.05)	2.32 (1.76 – 2.98)	232.42 (175.99 – 298.40)
Range	23 – 246	0.23 – 2.46	0.51 - 5.33	1.17 - 4.65	117.39 – 465.22
Geometric mean concentration (95% CI)	62.57 (53.91 – 72.63)	0.63 (0.54 - 0.73)	1.49 (1.28 – 1.73)	2.34 (2.09 – 2.61)	233.56 (208.97 – 261.05
Emtricitabine pharmacokinetics (n=43)					
Undetectable samples (%)	4/43 (17%)	4/43 (17%)	4/43 (9%)	4/43 (17%)	4/43 (17%)
Median (IQR)	139 (109 – 166)	5.56 (4.36 – 6.64)	63.5 (47.4 – 102)	53.45 (39.23 - 67.55)	1336.21 (980.77 – 1688.74)
Range	39.3 – 560	1.57 – 22.4	1.45 – 167	20.75 - 200.72	518.67 – 5017.9
Geometric mean concentration (95% CI)	133.68 (112.14 – 159.35)	5.35 (4.49 – 6.37)	55.95 (41.74 – 74.99)	52.78 (46.01 – 60.54)	1319.46 (1150.37 – 1513.41)

CSF = cerebrospinal fluid; IQR = interquartile range; 95% CI = 95% confidence interval

Table 3. Genetic associations with detectable log_{10}-transformed CSF-to-plasma EFV concentrations in South African adults.[†]

Chromo-some	Gene	Polymorphism (minor allele)	MAF	Univariate analysis		516G→T adjusted		516G→T & 983T→C adjusted		516G→T & 983T→C & *CYP2B6* 15582C→T adjusted	
				β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
13	*ABCC4*	rs9584273 (T)	0.07	0.14 (0.05 to 0.22)	2.3×10^{-03}	0.14 (0.06 to 0.22)	2.2×10^{-03}	0.14 (0.05 to 0.22)	2.8×10^{-03}	0.14 (0.06 to 0.22)	2.5×10^{-03}
13	*ABCC4*	rs9590160 (A)	0.09	-0.12 (-0.19 to -0.04)	2.9×10^{-03}	-0.12 (-0.19 to -0.04)	3.2×10^{-03}	-0.12 (-0.19 to -0.04)	3.5×10^{-03}	-0.12 (-0.19 to -0.04)	4.3×10^{-03}
13	*ABCC4*	rs74107818 (G)	0.10	-0.11 (-0.18 to -0.04)	3.0×10^{-03}	-0.11 (-0.18 to -0.04)	3.2×10^{-03}	-0.11 (-0.19 to -0.04)	3.3×10^{-03}	-0.11 (-0.18 to -0.04)	4.0×10^{-03}
13	*ABCC4*	rs74107809 (A)	0.07	-0.13 (-0.21 to -0.04)	5.3×10^{-03}	-0.13 (-0.21 to -0.04)	5.8×10^{-03}	-0.13 (-0.21 to -0.04)	4.8×10^{-03}	-0.13 (-0.21 to -0.04)	5.7×10^{-03}
19	*CYP2B6*	rs8100458 (C)	0.19	0.07 (0.02 to 0.12)	6.8×10^{-03}	0.07 (0.02 to 0.12)	6.8×10^{-03}	0.08 (0.03 to 0.14)	6.4×10^{-03}	0.10 (0.03 to 0.16)	5.4×10^{-03}
3	*ABCC5*	rs7610724 (G)	0.07	0.10 (0.03 to 0.17)	7.6×10^{-03}	0.10 (0.03 to 0.18)	7.7×10^{-03}	0.10 (0.03 to 0.17)	7.6×10^{-03}	0.11 (0.03 to 0.18)	9.8×10^{-03}
7	*ABCB1*	rs2235023 (T)	0.42	-0.06 (-0.10 to -0.02)	8.1×10^{-03}	-0.06 (-0.11 to -0.02)	7.8×10^{-03}	-0.06 (-0.11 to -0.02)	7.6×10^{-03}	-0.06 (-0.11 to -0.02)	9.2×10^{-03}
19	*CYP2G1P*	rs142357867 (T)	0.03	-0.16 (-0.28 to -0.04)	8.1×10^{-03}	-0.16 (-0.28 to -0.05)	8.9×10^{-03}	-0.17 (-0.29 to -0.06)	5.5×10^{-03}	-0.18 (-0.30 to -0.06)	5.1×10^{-03}
13	*ABCC4*	rs200689258 (AC)	0.09	0.11 (0.03 to 0.18)	8.2×10^{-03}	0.11 (0.03 to 0.19)	8.0×10^{-03}	0.11 (0.03 to 0.18)	1.1×10^{-02}	0.11 (0.03 to 0.19)	1.2×10^{-02}
3	*ABCC5*	rs7427051(A)	0.24	-0.07 (-0.11 to -0.02)	8.6×10^{-03}	-0.07 (-0.11 to -0.02)	8.6×10^{-03}	-0.07 (-0.12 to -0.02)	1.1×10^{-02}	-0.07 (-0.12 to -0.02)	1.2×10^{-02}
13	*ABCC4*	rs73548889 (C)	0.06	0.13 (0.05 to 0.22)	9.5×10^{-03}	0.13 (0.04 to 0.22)	9.9×10^{-03}	0.13 (0.03 to 0.22)	1.1×10^{-02}	0.14 (0.05 to 0.24)	6.9×10^{-03}
13	*ABCC4*	rs9524925 (G)	0.17	-0.07 (-0.12 to -0.02)	1.0×10^{-02}	-0.07 (-0.12 to -0.02)	7.6×10^{-03}	-0.07 (-0.13 to -0.02)	1.1×10^{-02}	-0.07 (-0.12 to -0.02)	1.0×10^{-02}
19	*CYP2B6*	Composite *CYP2B6* 516/983 (C)	0.41	-0.02 (-0.06 to 0.03)	0.47	NA	NA	NA	NA	NA	NA
19	CYP2B6	*CYP2B6* 516G→T§	0.29	-0.01 (-0.07 to 0.04)	0.62	NA	NA	NA	NA	NA	NA
19	CYP2B6	*CYP2B6* 983T→C*	0.13	0.02 (-0.10 to 0.06)	0.62	-0.03 (-0.10 to 0.06)	0.60	NA	NA	NA	NA
19	CYP2B6	*CYP2B6* 15582C→T*	0.10	-0.02 (-0.10 to 0.07)	0.72	-0.03 (-0.10 to 0.07)	0.68	-0.03 (-0.12 to 0.07)	0.57	NA	NA
19	CYP2A6	*CYP2A6* -48A→C*	0.09	-0.00 (-0.09 to 0.09)	0.96	-0.00 (-0.09 to 0.09)	0.97	0.01 (-0.09 to 0.12)	0.78	0.01 (-0.09 to 0.12)	0.80

[†]The targeted SNPs (*CYP2B6* 516G→T, *CYP2A6* -48A→C, *CYP2B6* 983T→C, *CYP2B6* 15582C→T, *SLCO1B1* 521T→ C and *SLCO1B1*) included 47 patients and the rest 43 patients; CSF = cerebrospinal fluid; EFV = efavirenz; MAF = minor alele frequency *SNP of interest but did not meet criteria of p-value<0.01; Bonferroni corrected *P*-value 5.68×10^{-05}; [§]*P*-value <0.05 accepted as significant for SNPs with a previously described association; NA= Not applicable

Table 4. Genetic associations with detectable log_{10}-transformed CSF-to-plasma 8-OH-EFV concentrations in 16 South African adults.

Chromo-some	Gene	Polymorphism (minor allele)	MAF	Univariate analysis		516G→T adjusted		516G→T & 983T→C adjusted		516G→T & 983T→C & *CYP2B6* 15582C→T adjusted	
				β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
3	*ABCC5*	rs6762938 (T)	0.31	0.23 (0.14 to 0.32)	2.6×10^{-04}	0.23 (0.14 to 0.33)	3.9×10^{-04}	0.22 (0.12 to 0.32)	1.1×10^{-03}	0.22 (0.11 to 0.33)	2.1×10^{-02}
13	*ABCC4*	rs11343244 (T)	0.10	0.41 (0.24 to 0.58)	3.8×10^{-04}	0.41 (0.23 to 0.59)	6.2×10^{-04}	0.39 (0.20 to 0.49)	1.1×10^{-03}	0.41 (0.23 to 0.59)	8.5×10^{-04}
13	*ABCC4*	rs7997839 (G)	0.14	0.41 (0.24 to 0.58)	3.8×10^{-04}	0.41 (0.23 to 0.59)	6.2×10^{-04}	0.39 (0.20 to 0.49)	1.1×10^{-03}	0.41 (0.23 to 0.59)	8.5×10^{-04}
3	*ABCC5*	rs10937161 (T)	0.20	0.34 (0.19 to 0.49)	4.7×10^{-04}	0.36 (0.21 to 0.51)	4.1×10^{-04}	0.36 (0.22 to 0.49)	2.3×10^{-04}	0.35 (0.21 to 0.50)	5.0×10^{-04}
3	*ABCC5*	rs36092077 (A)	0.16	0.34 (0.17 to 0.51)	1.8×10^{-03}	0.39 (0.21 to 0.56)	8.9×10^{-04}	0.39 (0.23 to 0.54)	3.7×10^{-04}	0.39 (0.21 to 0.56)	7.1×10^{-04}
3	*ABCC5*	rs6807271 (A)	0.31	0.21 (0.10 to 0.31)	1.8×10^{-03}	0.21 (0.10 to 0.32)	2.6×10^{-03}	0.21 (0.10 to 0.32)	2.6×10^{-03}	0.20 (0.08 to 0.31)	5.8×10^{-03}
3	*ABCC5*	rs59309690 (A)	0.09	0.58 (0.26 to 0.90)	3.2×10^{-03}	0.60 (0.27 to 0.93)	3.7×10^{-03}	0.58 (0.26 to 0.90)	4.1×10^{-03}	0.57 (0.24 to 0.90)	6.5×10^{-03}
4	*ABCG2*	rs2728108 (A)	0.08	0.58 (0.26 to 0.90)	3.2×10^{-03}	0.60 (0.27 to 0.93)	3.7×10^{-03}	0.58 (0.26 to 0.90)	4.1×10^{-03}	0.57 (0.24 to 0.90)	6.5×10^{-03}
13	*ABCC4*	rs1678392 (A)	0.19	0.32 (0.14 to 0.50)	3.9×10^{-03}	0.32 (0.14 to 0.51)	4.9×10^{-03}	0.30 (0.11 to 0.50)	1.1×10^{-02}	0.31 (0.11 to 0.51)	1.1×10^{-02}
13	*ABCC4*	rs116336902 (A)	0.07	0.32 (0.14 to 0.50)	4.1×10^{-03}	0.32 (0.13 to 0.51)	5.1×10^{-03}	0.30 (0.10 to 0.50)	1.1×10^{-02}	0.31 (0.11 to 0.51)	1.1×10^{-02}
13	*ABCC4*	rs147385814 (C)	0.07	0.32 (0.14 to 0.50)	4.1×10^{-03}	0.32 (0.13 to 0.51)	5.1×10^{-03}	0.30 (0.10 to 0.50)	1.1×10^{-02}	0.31 (0.11 to 0.51)	1.1×10^{-02}
13	*ABCC4*	rs4771904 (T)	0.27	-0.27 (-0.43 to -0.10)	6.4×10^{-03}	-0.27 (-0.44 to -0.10)	7.8×10^{-03}	-0.26 (-0.45 to -0.07)	2.2×10^{-02}	-0.27 (-0.44 to -0.10)	3.3×10^{-02}
3	*ABCC5*	rs6794223 (G)	0.14	0.25 (0.10 to 0.41)	6.8×10^{-03}	0.27 (0.10 to 0.43)	6.7×10^{-03}	0.25 (0.09 to 0.41)	9.0×10^{-03}	0.25 (0.08 to 0.42)	1.4×10^{-02}
11	*SLCO2B1*	rs57141326 (A)	0.08	0.34 (0.13 to 0.55)	7.2×10^{-03}	0.34 (0.12 to 0.56)	9.6×10^{-03}	0.31 (0.09 to 0.54)	1.8×10^{-02}	0.33 (0.11 to 0.56)	1.5×10^{-02}
7	*ABCB1*	rs28401781 (T)	0.24	0.18 (0.07 to 0.30)	8.7×10^{-03}	0.19 (0.07 to 0.32)	9.0×10^{-03}	0.19 (0.06 to 0.31)	5.8×10^{-03}	0.22 (0.11 to 0.33)	2.4×10^{-03}
3	*ABCC5*	rs56889675 (T)	0.26	0.21 (0.08 to 0.35)	8.8×10^{-03}	0.24 (0.09 to 0.38)	7.6×10^{-03}	0.27 (0.14 to 0.39)	1.1×10^{-03}	0.26 (0.13 to 0.39)	2.1×10^{-03}
3	*ABCC5*	rs10470524 (T)	0.22	0.21 (0.08 to 0.35)	8.8×10^{-03}	0.24 (0.09 to 0.38)	7.6×10^{-03}	0.27 (0.14 to 0.39)	1.1×10^{-03}	0.26 (0.13 to 0.39)	2.1×10^{-03}
13	*ABBC4*	rs4148551 (T)	0.36	-0.23 (-0.38 to -0.08)	8.9×10^{-03}	-0.25 (-0.41 to -0.10)	7.4×10^{-03}	-0.23 (-0.39 to -0.08)	1.1×10^{-02}	-0.23 (-0.40 to -0.07)	1.8×10^{-03}
19	*CYP2B6*	Composite *CYP2B6* 516/983	0.41	-0.07 (-0.23 to 0.09)	0.42	NA	NA	NA	NA	NA	NA
19	*CYP2B6*	*CYP2B6* 516G→T§	0.29	-0.00 (-0.17 to 0.17)	0.99	NA	NA	NA	NA	NA	NA
19	*CYP2B6*	*CYP2B6* 983T→C*	0.13	-0.17 (-0.42 to 0.08)	0.19	-0.18 (-0.45 to 0.08)	0.19	NA	NA	NA	NA
19	*CYP2B6*	*CYP2B6* 15582C→T*	0.10	0.02 (-0.22 to 0.25)	0.88	0.02 (-0.24 to 0.28)	0.88	-0.05 (-0.33 to 0.22)	0.70	NA	NA
19	*CYP2A6*	*CYP2A6* -48A→C*	0.09	-0.03 (-0.30 to 0.23)	0.80	-0.04 (-0.32 to 0.25)	0.81	0.10 (-0.23 to 0.43)	0.55	0.09 (-0.26 to 0.52)	0.61

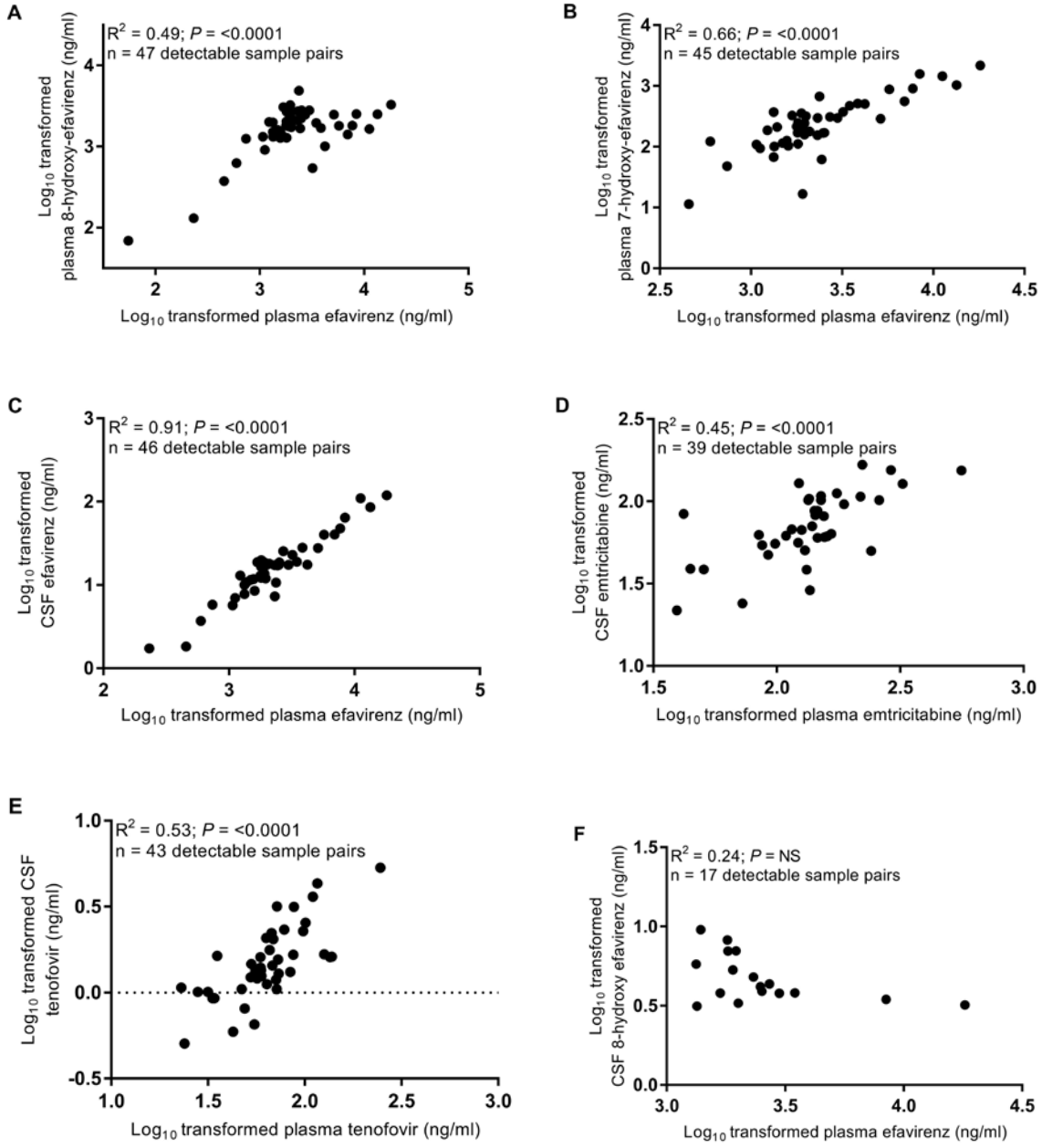
CSF = cerebrospinal fluid; EFV = efavirenz; 8-OH-EFV = 8-hydroxy-efavirenz; MAF = minor alele frequency *SNP of interest but did not meet criteria of p-value<0.01; Bonferroni corrected *P*-value 5.68×10^{-05}; §*P*-value <0.05 accepted as significant for SNPs with a previously described association; NA= Not applicable

Table 5. EFV metaboliser status and detectable EFV, 8-OH-EFV and 7-OH-EFV concentrations in CSF and plasma respectively**.**

		Geometric mean concentration (95% CI)							
		Plasma (ng/mL)					**Cerebrospinal fluid (ng/mL)**		
Metaboliser genotype	**Participants (%)**	**EFV**	**8-OH-EFV**	**8-OH-EFV/EFV**	**7-OH-EFV** (n=45/47 detectable)	**7-OH-EFV/EFV**	**EFV** (n=46/47 detectable)	**8-OH-EFV** (n=17/47 detectable)	**8-OH-EFV/EFV**
Slow	9 (19.1)	6896.9 (3984.1 - 11939.4)**	1860.2 (1421.3 - 2434.6)	0.27 (0.17 – 0.42)**	810.7** (466.3 - 1409.6)**	0.12 (0.10 – 0.14)	45.8 (25.0 - 83.9)**	1.8 (1.4 - 2.4)	0.04 (0.0 – 3.17)
Intermediate	21 (44.7)	1878.1 (1371.4 - 2572.0)	1543.8 (1091.4 - 2183.8)	0.82 (0.64 – 1.06)	185.5 (108.0 - 318.5)	0.11 (0.08 – 0.15)	12.7 (9.3 - 17.4)	2.7 (2.0 - 3.5)	0.36 (0.24 – 0.54)
Extensive	17 (34.7)	1253.48 (778.9 - 2017.2)	1467.1 (907.9 - 2370.6)	1.17 (1.03 – 1.33)	89.6 (45.4 – 176.7)	0.07 (0.05 – 0.11)	9.0 (5.0 - 15.9)	2.2 (1.6 - 3.0)	0.28 (0.14 – 0.56)
P value†		<0.01	NS	<0.01	<0.01	NS	<0.01	NS	NS

CSF = cerebrospinal fluid; EFV = efavirenz, 7-OH-EFV = 7-hydroxy-efavirenz; 8-OH-EFV = 8-hydroxy-efavirenz; †*P* values were determined by One-way Analysis of Variance (ANOVA); NS = not significant; ** = p <0.01

Figure 1. Pearson correlation plots for log_{10}-transformed plasma and cerebrospinal fluid (CSF) concentrations for efavirenz and metabolites, emtricitabine and tenofovir in panels A-H. The relationship between **(A)** log_{10} transformed plasma efavirenz concentrations and plasma 8-hydroxy-efavirenz concentrations **(B)** log_{10} transformed plasma efavirenz concentrations and plasma 7-hydroxy-efavirenz concentrations **(C)** log_{10} transformed plasma efavirenz concentrations and log_{10} transformed cerebrospinal fluid (CSF) efavirenz concentrations **(D)** log_{10} transformed plasma emtricitabine and CSF emtricitabine **(E)** log_{10} transformed plasma tenofovir and CSF tenofovir **(F)** log_{10} transformed plasma efavirenz concentrations and detectable CSF 8-hydroxy-efavirenz concentrations **(G)** log_{10} transformed plasma 8-hydroxy-efavirenz and CSF 8-hydroxy-efavirenz **(H)** log_{10} transformed plasma 8-hydroxy-efavirenz and 7-hydroxy-efavirenz. NS = not significant.



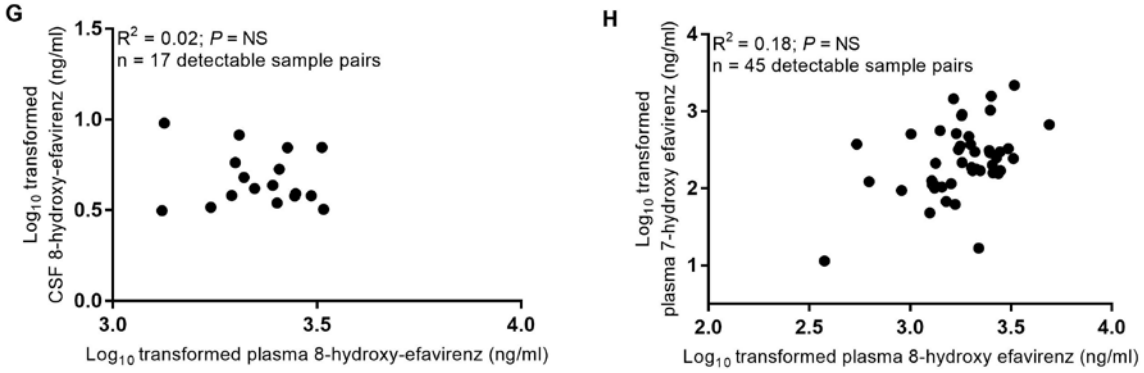

G
R² = 0.02; P = NS
n = 17 detectable sample pairs
Log10 transformed CSF 8-hydroxy-efavirenz (ng/ml)
Log10 transformed plasma 8-hydroxy-efavirenz (ng/ml)
1.5
1.0
0.5
0.0
3.0
3.5
4.0
H
R² = 0.18; P = NS
n = 45 detectable sample pairs
Log10 transformed plasma 7-hydroxy efavirenz (ng/ml)
Log10 transformed plasma 8-hydroxy efavirenz (ng/ml)
4
3
2
1
2.0
2.5
3.0
3.5
4.0

Figure 2. Cerebrospinal fluid (CSF)-to-plasma concentration ratios of detectable pairs of plasma and CSF samples versus time after dosing. The lines are linear regression lines and were not statistically significant for any ratio, including 8-OH-EFV (p=0.09). EFV = efavirenz; 8-OH-EFV = 8-hydroxy-efavirenz; TFV = tenofovir; FTC = emtricitabine

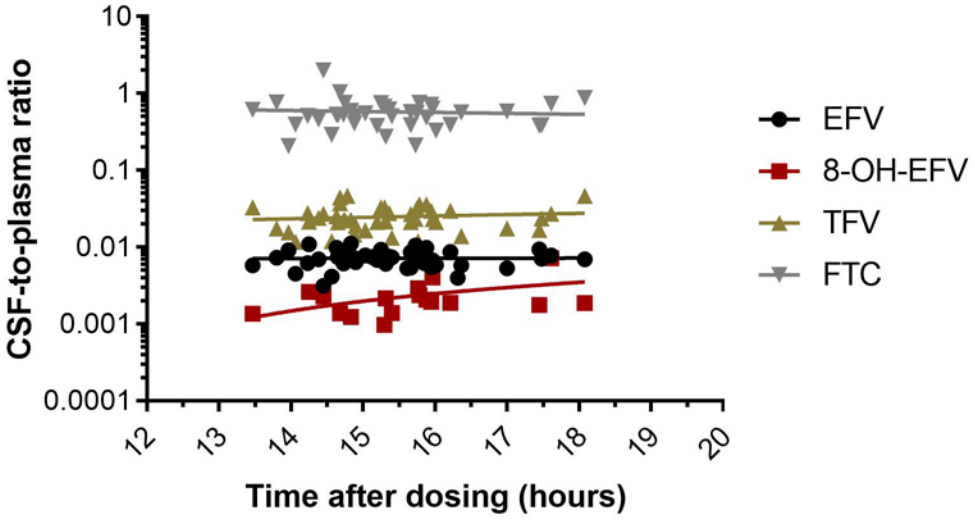
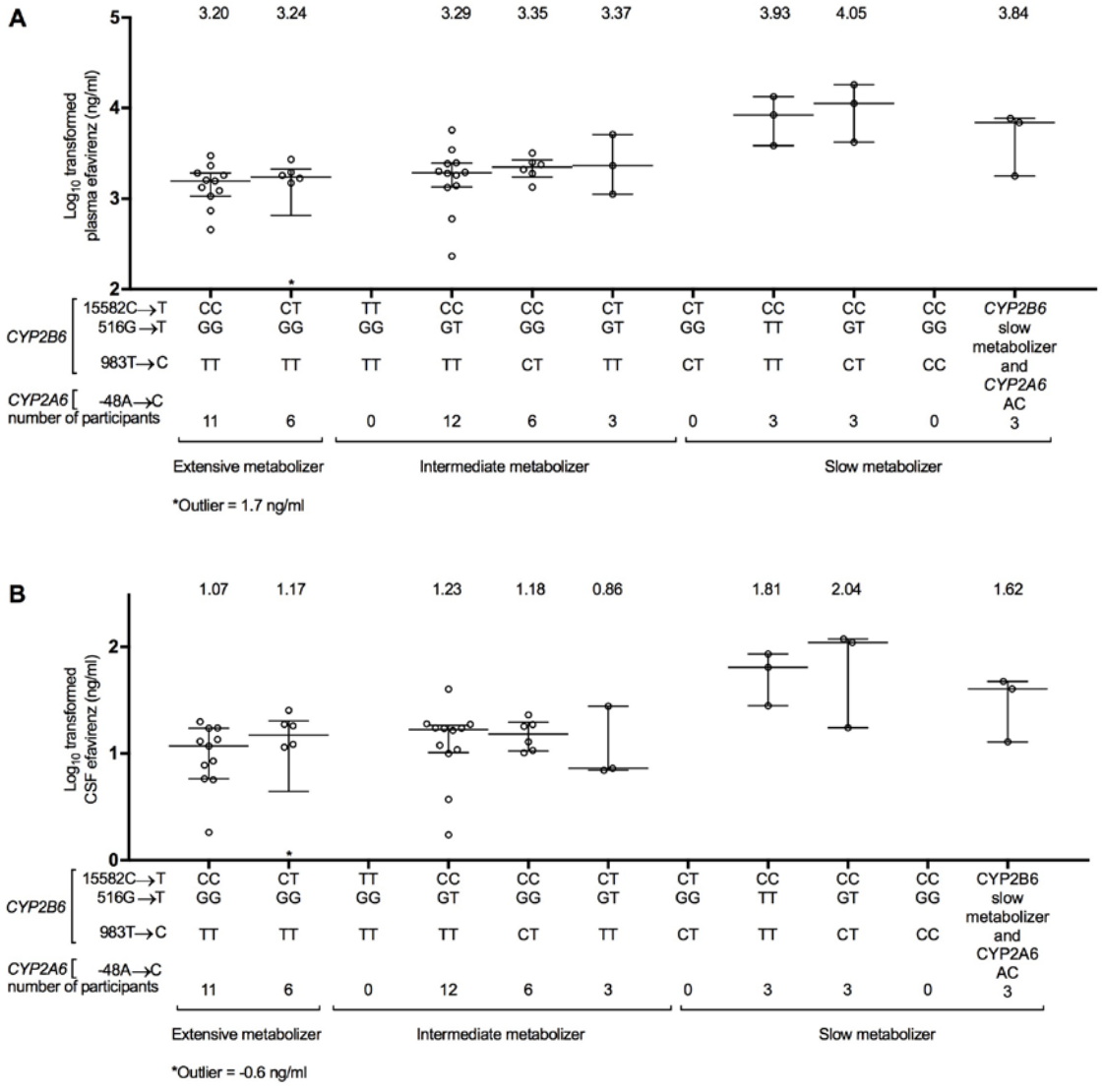
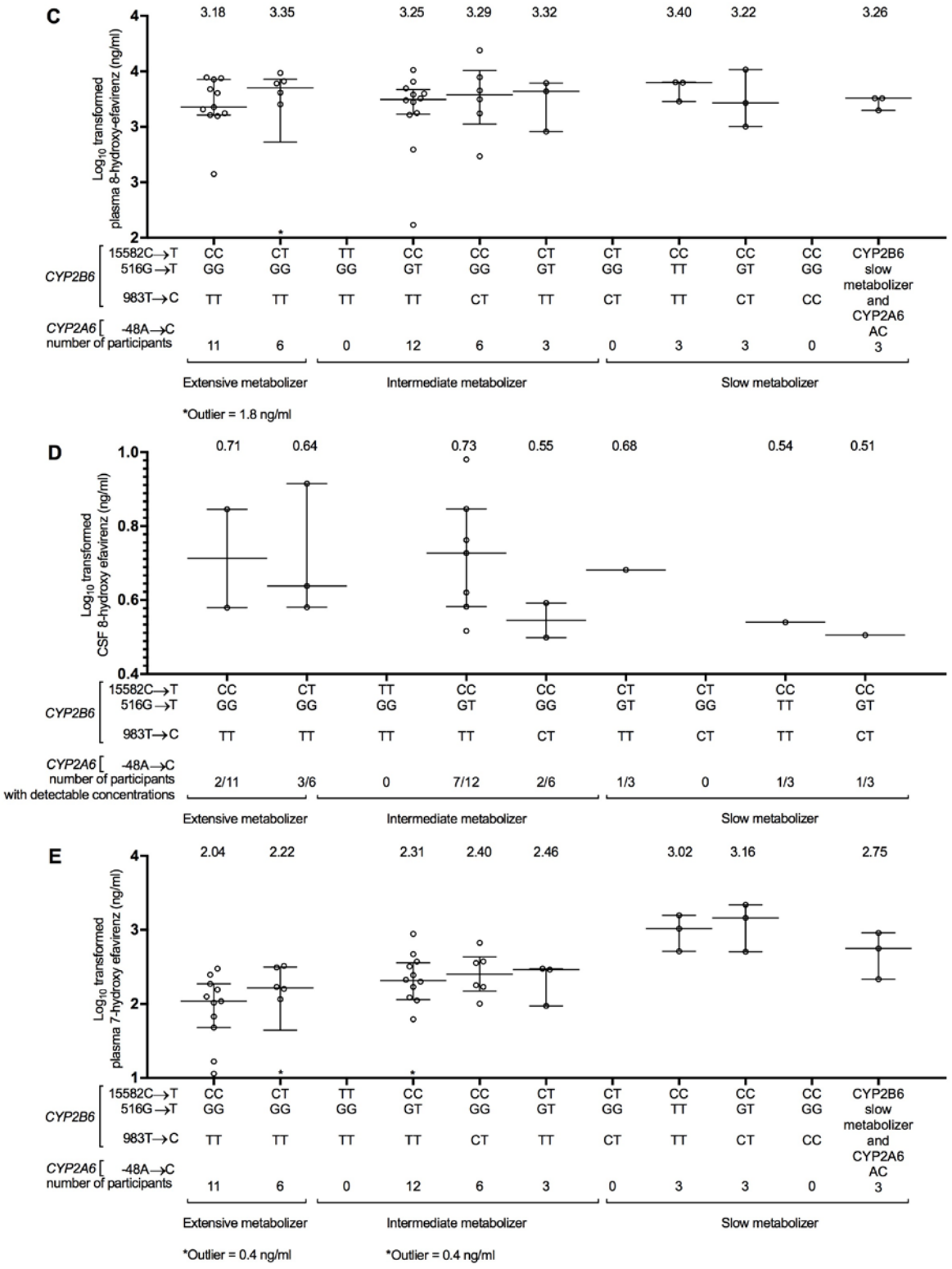


Figure 3. Relationships between efavirenz *CYP2B6* metabolizer status and log_{10}-transformed efavirenz and metabolites concentrations in plasma or cerebrospinal fluid (A) Relationship between *CYP2B6*/*CYP2A6* polymorphisms and log_{10} transformed plasma efavirenz concentrations, **(B)** *CYP2B6*/*CYP2A6* polymorphisms and cerebrospinal fluid efavirenz concentrations **(C)** *CYP2B6*/*CYP2A6* polymorphisms and plasma 8-hydroxy-efavirenz concentrations **(D)** *CYP2B6*/*CYP2A6* polymorphisms and cerebrospinal fluid 8-hydroxy-efavirenz and **(E)** *CYP2B6*/*CYP2A6* polymorphisms and plasma 7-hydroxy-efavirenz concentrations. The x-axes show *CYP2B6*/*CYP2A6* haplotypes, number of participants with each haplotype and efavirenz metabolizer status (extensive, intermediate or slow). Median concentrations are at the top. Error bars indicate medians and interquartile ranges.




C
Log10 transformed plasma 8-hydroxy-efavirenz (ng/ml)
3.18 3.35 3.25 3.29 3.32 3.40 3.22 3.26
CYP2B6
15582C→T CC CT TT CC CC CT CT CC CC CC CYP2B6 slow metabolizer and CYP2A6 AC
516G→T GG GG GG GT GG GT GG TT GT GG
983T→C TT TT TT TT CT TT CT TT CT CC
CYP2A6 -48A→C
number of participants 11 6 0 12 6 3 0 3 3 0 3
Extensive metabolizer
Intermediate metabolizer
Slow metabolizer
*Outlier = 1.8 ng/ml
D
Log10 transformed CSF 8-hydroxy efavirenz (ng/ml)
0.71 0.64 0.73 0.55 0.68 0.54 0.51
CYP2B6
15582C→T CC CT TT CC CC CT CT CC CC
516G→T GG GG GG GT GG GT GG TT GT
983T→C TT TT TT TT CT TT CT TT CT
CYP2A6 -48A→C
number of participants with detectable concentrations 2/11 3/6 0 7/12 2/6 1/3 0 1/3 1/3
Extensive metabolizer
Intermediate metabolizer
Slow metabolizer
E
Log10 transformed plasma 7-hydroxy efavirenz (ng/ml)
2.04 2.22 2.31 2.40 2.46 3.02 3.16 2.75
CYP2B6
15582C→T CC CT TT CC CC CT CT CC CC CC CYP2B6 slow metabolizer and CYP2A6 AC
516G→T GG GG GG GT GG GT GG TT GT GG
983T→C TT TT TT TT CT TT CT TT CT CC
CYP2A6 -48A→C
number of participants 11 6 0 12 6 3 0 3 3 0 3
Extensive metabolizer
Intermediate metabolizer
Slow metabolizer
*Outlier = 0.4 ng/ml
*Outlier = 0.4 ng/ml

Supplementary material: tables

Table S1. Genetic associations with detectable log_{10}-transformed plasma efavirenz, 8-hydroxy-efavirenz and 7-hydroxy-efavirenz concentrations in South African adults.[†]

Plasma efavirenz		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
CYP2B6	Composite *CYP2B6* 516/983	**0.34 (0.20 to 0.48)**	**1.7 x10^{-05}**	NA		NA	
CYP2B6	rs60618302	**-0.43 (-0.62 to -0.25)**	**5.3 x10^{-05}**	-0.36 (-0.61 to -0.12)	5.6 x10^{-03}	-0.26 (-0.51 to -0.03)	3.6 x10^{-02}
ABCB1	rs115780656	-0.45 (-0.70 to -0.20)	1.1 x10^{-03}	-0.42 (-0.61 to -0.12)	5.8 x10^{-04}	-0.37 (-0.58 to -0.17)	9.7 x10^{-04}
CYP2B6	rs73557157	-0.38 (-0.60 to -0.16)	1.6 x10^{-03}	-0.24 (-0.52 to 0.04)	9.7 x10^{-02}	-0.12 (-0.40 to 0.15)	0.38
CYP2B6	*CYP2B6* 516G→T[§]	**0.30 (0.12 to 0.49)**	**2.0 x10^{-03}**	NA		NA	
CYP2B6	rs59243457	0.31 (0.12 to 0.49)	2.7 x10^{-03}	0.13 (-0.34 to 0.61)	0.59	0.25 (-0.18 to 0.69)	0.26
ABCC4	rs72643607	-0.60 (-0.98 to -0.22)	3.3 x10^{-03}	-0.49 (-0.85 to -0.13)	1.1 x10^{-02}	-0.46 (-0.78 to -0.14)	7.4 x10^{-03}
CYP2B6	rs56164728	-0.53 (-0.88 to -0.17)	5.7 x10^{-03}	-0.40 (-0.74 to -0.06)	2.8 x10^{-02}	-0.36 (-0.67 to -0.05)	3.0 x10^{-02}
ABCC5	rs56889675	-0.27 (-0.46 to -0.08)	7.3 x10^{-03}	-0.20 (-0.38 to -0.02)	3.5 x10^{-02}	-0.17 (-0.34 to -0.01)	5.0 x10^{-02}
CYP2B6	*CYP2B6* 983T→C[§]	**0.35 (0.08 to 0.62)**	**1.4 x10^{-02}**	**0.38 (0.14 to 0.61)**	**2.7 x10^{-03}**	NA	
CYP2B6	*CYP2B6* 15582C→T*	-0.23 (-0.54 to 0.08)	0.15	-0.14 (-0.43 to 0.15)	0.35	-0.00 (-0.29 to 0.28)	0.98
CYP2A6	*CYP2A6* -48A→C*	0.02 (-0.31 to 0.35)	0.91	-0.00 (-0.29 to -0.29)	1.00	-0.31 (-0.62 to -0.00)	0.05
Plasma 8-hydroxy-efavirenz		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
ABCB1	rs115780656	-0.36 (-0.55 to -0.18)	4.3 x10^{-04}	-0.36 (-0.55 to -0.17)	5.2 x10^{-04}	-0.35 (-0.55 to -0.16)	8.8 x10^{-04}
ABCG2	rs45621036	-0.30 (-0.46 to -0.14)	5.9 x10^{-04}	-0.30 (-0.46 to -0.14)	6.3 x10^{-04}	-0.30 (-0.46 to -0.13)	9.7 x10^{-04}
ABCG2	rs2231159	-0.28 (-0.44 to -0.13)	8.0 x10^{-04}	-0.29 (-0.44 to -0.13)	8.9 x10^{-04}	-0.28 (-0.44 to -0.13)	9.2 x10^{-04}
CYP2G1P	rs142357867	-0.64 (-0.99 to -0.29)	9.1 x10^{-04}	-0.64 (-1.00 to -0.29)	1.0 x10^{-03}	-0.63 (-0.99 to -0.26)	1.6 x10^{-03}
ABCB1	rs57924923	-0.22 (-0.36 to -0.08)	2.4 x10^{-03}	-0.23 (-0.36 to -0.09)	2.3 x10^{-03}	-0.23 (-0.36 to -0.09)	2.5 x10^{-03}
ABCB1	rs2235023	-0.21 (-0.35 to -0.08)	3.5 x10^{-03}	-0.22 (-0.36 to -0.09)	3.0 x10^{-03}	-0.22 (-0.36 to -0.08)	3.1 x10^{-03}

ABCG2	rs2622614	-0.22 (-0.36 to -0.08)	3.5 x10^{-03}	-0.22 (-0.36 to -0.08)	3.5 x10^{-03}	-0.23 (-0.37 to -0.09)	2.6 x10^{-03}
CYP2A6	rs16974537	-0.49 (-0.81 to -0.17)	4.6 x10^{-03}	-0.50 (-0.82 to -0.17)	4.6 x10^{-03}	-0.50 (-0.82 to -0.18)	4.2 x10^{-03}
ABCC4	rs72643607	-0.44 (-0.73 to -0.15)	4.6 x10^{-03}	-0.46 (-0.76 to -0.16)	4.7 x10^{-03}	-0.45 (-0.75 to -0.15)	5.4 x10^{-03}
CYP2A6	rs56164728	-0.38 (-0.65 to -0.11)	9.0 x10^{-03}	-0.40 (-0.69 to -0.12)	8.6 x10^{-03}	-0.39 (-0.68 to -0.10)	1.1 x10^{-02}
ABCC4	rs73557775	-0.27 (-0.47 to -0.08)	9.2 x10^{-03}	-0.27 (-0.47 to -0.07)	1.0 x10^{-02}	-0.28 (-0.48 to -0.08)	8.9 x10^{-02}
CYP2A7	rs149560129	-0.45 (-0.78 to -0.13)	9.2 x10^{-03}	-0.46 (-0.79 to -0.13)	9.2 x10^{-03}	-0.44 (-0.78 to -0.10)	1.4 x10^{-02}
CYP2B6	Composite *CYP2B6* 516/983*	0.05 (-0.08 to 0.18)	0.49	NA		NA	
CYP2B6	*CYP2B6* 516G→T*	0.03 (-0.13 to 0.19)	0.69	NA		NA	
CYP2B6	*CYP2B6* 983T→C*	0.07 (-0.15 to 0.29)	0.54	0.07 (-0.15 to -0.29)	0.53	NA	
CYP2B6	*CYP2B6* 15582C→T*	-0.06 (-0.31 to 0.19)	0.64	-0.05 (-0.30 to -0.20)	0.70	-0.03 (-0.30 to 0.24)	0.85
CYP2A6	*CYP2A6* -48A→C*	-0.15(-0.40 to 0.10)	0.26	-0.15 (-0.40 to 0.11)	0.26	-0.26 (-0.56 to -0.03)	0.09
Plasma 7-hydroxy-efavirenz		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
CYP2B6	Composite *CYP2B6* 516/983	**0.45 (0.10 to 0.66)**	**5.9 x10^{-05}**	NA	NA	NA	NA
CYP2A6	rs56164728	-1.02 (-1.47 to -0.57)	6.4 x10^{-05}	-0.89 (-1.33 to -0.44)	3.7 x10^{-04}	-0.83 (-1.23 to -0.43)	2.4 x10^{-04}
ABCB1	rs115780656	-0.75 (-1.08 to -0.42)	6.8 x10^{-05}	**-0.71 (-1.01 to -0.42)**	**2.9 x10^{-05}**	**-0.65 (-0.92 to -0.37)**	**4.1 x10^{-05}**
CYP2B6	rs60618302	-0.56 (-0.84 to -0.28)	2.9 x10^{-04}	-0.46 (-0.82 to -0.11)	1.5 x10^{-02}	-0.33 (-0.68 to -0.03)	0.08
CYP2B6	rs2279345	-0.54 (-0.82 to -0.27)	3.7 x10^{-04}	-0.44 (-0.73 to -0.14)	6.0 x10^{-03}	-0.35 (-0.63 to -0.06)	2.2 x10^{-02}
CYP2A6	rs10853742	-0.52 (-0.81 to -0.23)	1.2 x10^{-03}	-0.45 (-0.72 to -0.17)	3.0 x10^{-03}	**-0.55 (-0.78 to -0.32)**	**3.5 x10^{-05}**
CYP2A6	rs7248240	-0.67 (-1.07 to -0.27)	2.2 x10^{-03}	-0.55 (-0.94 to -0.16)	9.0 x10^{-03}	-0.51 (-0.87 to -0.16)	7.3 x10^{-03}
CYP2A6	rs7251418	-0.69 (-1.10 to -0.27)	2.5 x10^{-03}	-0.58 (-0.98 to -0.18)	712 x10^{-03}	-0.56 (-0.92 to -0.20)	3.9 x10^{-03}
ABCC4	rs72643607	-0.85 (-1.38 to -0.32)	3.3 x10^{-03}	-0.71 (-1.22 to -0.19)	1.1 x10^{-02}	-0.67 (-1.14 to -0.21)	7.4 x10^{-03}
CYP2B6	rs73557157	-0.50 (-0.81 to -0.19)	3.3 x10^{-03}	-0.33 (-0.74 to 0.07)	0.11	-0.17 (-0.56 to 0.23)	0.41
ABCB1	rs57924923	-0.38 (-0.64 to -0.13)	5.2 x10^{-03}	-0.43 (-0.65 to -0.21)	4.7 x10^{-04}	-0.42 (-0.62 to -0.23)	1.2 x10^{-04}
CYP2B6	rs59243457	0.40 (0.13 to 0.67)	5.4 x10^{-03}	0.22 (-0.47 to 0.90)	0.54	0.38 (-0.24 to 1.01)	0.24

CYP2B6	*CYP2B6* 516G→T[§]	**0.39 (0.14 to 0.70)**	**5.5 x10^{-03}**	NA	NA	NA	NA
CYP2B6	*CYP2B6* 983T→C[§]	**0.50 (0.12 to 0.86)**	**1.5 x10^{-02}**	**0.59 (0.22 to 0.96)**	**3.7 x10^{-03}**	NA	NA
CYP2B6	*CYP2B6* 15582C→T*	-0.23 (-0.68 to 0.21)	0.31	-0.11 (-0.53 to -0.31)	0.61	0.09 (-0.35 to 0.53)	0.67
CYP2A6	CYP2A6 -48A→C*	-0.09 (-0.55 to 0.38)	0.71	-0.11 (-0.54 to -0.32)	0.62	**-0.59 (-1.01 to -0.16)**	**1.0 x10^{-02}**

[†]The targeted SNPs (*CYP2B6* 516G→T, *CYP2A6* -48A→C, *CYP2B6* 983T→C, *CYP2B6* 15582C→T, *SLCO1B1* 521T→ C and *SLCO1B1*) included 47 patients and the rest 43 patients; *SNP of interest but did not meet criteria of p-value<0.01; Bonferroni corrected *P*-value 5.68 x10^{-05}; [§]*P*-value <0.05 accepted as significant for SNPs with a previously described association; NA= Not applicable

Table S2. Genetic associations with detectable log_{10}-transformed cerebrospinal fluid (CSF) efavirenz and 8-hydroxy efavirenz concentrations in South African adults.[†]

CSF efavirenz		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
CYP2B6	Composite *CYP2B6* 516/983	**0.33 (0.17 to 0.48)**	**1.7 x10^{-04}**	NA	NA	NA	NA
ABCB1	rs115780656	-0.51 (-0.77 to -0.26)	3.4 x10^{-04}	-0.50 (-0.72 to 0.26)	1.8 x10^{-04}	-0.45 (-0.67 to 0.22)	3.3 x10^{-04}
CYP2B6	rs60618302	-0.40 (-0.61 to -0.19)	5.7 x10^{-04}	-0.31 (-0.58 to -0.04)	3.0 x10^{-02}	-0.22 (-0.49 to 0.06)	0.16
ABCB1	rs57924923	-0.30 (0.49 to -0.12)	3.4 x10^{-03}	-0.34 (0.50 to -0.18)	2.1 x10^{-04}	-0.33 (0.50 to -0.18)	7.5 x10^{-05}
CYP2B6	rs59243457	0.31 (0.11 to 0.51)	4.0 x10^{-03}	0.18 (-0.34 to 0.69)	0.50	0.29 (-0.19 to 0.77)	0.24
ABCC4	rs72643607	-0.61 (-1.05 to -0.20)	5.5 x10^{-03}	-0.49 (-0.88 to -0.10)	1.8 x10^{-02}	-0.47 (-0.83 to -0.11)	1.5 x10^{-02}
CYP2B6	*CYP2B6* 516G→T	**0.29 (0.09 to 0.49)**	**6.2 x10^{-03}**	NA	NA	NA	NA
ABCC5	rs56889675	-0.39 (-0.64 to -0.14)	7.3 x10^{-03}	-0.21 (-0.41 to -0.02)	3.3 x10^{-02}	-0.19 (-0.38 to -0.01)	4.8 x10^{-02}
CYP2B6	rs56164728	-0.55 (-0.92 to -0.16)	7.5 x10^{-03}	-0.42 (-0.78 to -0.05)	3.3 x10^{-02}	-0.38 (-0.73 to -0.03)	4.0 x10^{-02}
ABCB1	rs2235023	-0.27 (-0.47 to -0.08)	8.1 x10^{-03}	-0.33 (-0.50 to -0.17)	3.2 x10^{-04}	-0.33 (-0.50 to -0.17)	1.1 x10^{-04}
CYP2B6	*CYP2B6* 983T→C[§]	**0.33 (0.04 to 0.61)**	**3.0 x10^{-02}**	**0.36 (0.10 to 0.62)**	**1.0 x10^{-02}**	NA	NA
CYP2B6	*CYP2B6* 15582C→T*	-0.24 (-0.58 to 0.09)	0.15	-0.16 (-0.48 to 0.16)	0.33	-0.03 (-0.35 to 0.29)	0.85
CYP2A6	CYP2A6 -48A→C*	0.02 (-0.33 to 0.37)	0.93	-0.00 (-0.33 to 0.32)	0.99	-0.30 (-0.64 to 0.05)	0.10
CSF 8-hydroxy-efavirenz (n=16)		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
ABCC5	rs6762938	0.18 (0.11 to 0.24)	7.8 x10^{-05}	0.18 (0.12 to 0.24)	9.8 x10^{-05}	0.16 (0.10 to 0.23)	2.3 x10^{-04}
ABCC5	rs6807271	0.17 (0.10 to 0.24)	3.4 x10^{-04}	0.17 (0.09 to 0.24)	6.2 x10^{-04}	0.16 (0.10 to 0.22)	2.7 x10^{-04}
ABCC5	rs6807670	0.14 (0.06 to 0.21)	2.9 x10^{-03}	0.14 (0.07 to 0.22)	2.9 x10^{-03}	0.14 (0.09 to 0.20)	3.2 x10^{-03}
ABCC4	rs11343244	0.27 (0.12 to 0.41)	3.1 x10^{-03}	0.27 (0.12 to 0.42)	4.2 x10^{-03}	0.24 (0.09 to 0.38)	7.3 x10^{-03}
ABCC4	rs7997839	0.27 (0.12 to 0.41)	3.1 x10^{-03}	0.27 (0.12 to 0.42)	4.2 x10^{-03}	0.24 (0.09 to 0.38)	7.3 x10^{-03}
SLCO2B1	rs2851079	-0.14 (-0.23 to -0.05)	6.9 x10^{-03}	-0.14 (-0.23 to -0.05)	9.5 x10^{-03}	-0.12 (-0.23 to -0.01)	0.05
ABCC4	rs9590177	0.20 (0.07 to 0.32)	8.2 x10^{-03}	0.20 (0.07 to 0.33)	1.0 x10^{-02}	0.17 (0.03 to 0.30)	3.4 x10^{-02}

ABCC5	rs6794223	0.18 (0.06 to 0.30)	9.1 x10^{-03}	0.19 (0.07 to 0.31)	7.4 x10^{-03}	0.18 (0.07 to 0.28)	6.7 x10^{-03}
ABCB1	rs2235067	0.22 (0.08 to 0.36)	9.5 x10^{-03}	0.24 (0.09 to 0.39)	8.8 x10^{-03}	0.24 (0.12 to 0.37)	2.4 x10^{-03}
ABCB1	rs10274587	0.22 (0.08 to 0.36)	9.5 x10^{-03}	0.24 (0.09 to 0.39)	8.8 x10^{-03}	0.24 (0.12 to 0.37)	2.4 x10^{-03}
CYP2B6	Composite *CYP2B6* 516/983	-0.08 (-0.19 to 0.04)	0.20	NA	NA	NA	NA
CYP2B6	*CYP2B6* 516G→T[§]	-0.01 (-0.14 to 0.11)	0.83	NA	NA	NA	NA
CYP2B6	*CYP2B6* 983T→C[§]	-0.17 (-0.34 to 0.00)	0.08	-0.19 (-0.37 to -0.00)	0.07	NA	NA
CYP2B6	*CYP2B6* 15582C→T*	0.04 (-0.13 to 0.21)	0.64	0.04 (-0.15 to 0.23)	0.68	-0.03 (-0.22 to 0.16)	0.75
CYP2A6	CYP2A6 -48A→C*	-0.03 (-0.22 to 0.16)	0.76	-0.04 (-0.24 to 0.17)	0.71	0.10 (-0.12 to 0.32)	0.40
CSF 8-hydroxy-efavirenz		Unadjusted analysis					
Gene	Polymorphism	OR (95%CI)	*P*-value				
ABCC5	rs35494670	0.13 (0.03 to 0.54)	4.8 x10^{-03}	NA	NA	NA	NA
ABCC5	rs11404217	0.18 (0.05 to 0.64)	8.1 x10^{-03}	NA	NA	NA	NA
ABCC4	rs1751046	0.21 (0.06 to 0.68)	9.7 x10^{-03}	NA	NA	NA	NA
CYP2B6	Composite *CYP2B6* 516/983	0.98 (0.43 to 2.33)	0.96	NA	NA	NA	NA
CYP2B6	*CYP2B6* 516G→T[§]	1.35 (0.51 to 3.58)	0.54	NA	NA	NA	NA
CYP2B6	*CYP2B6* 983T→C[§]	1.48 (0.34 to 6.47)	0.61	NA	NA	NA	NA
CYP2B6	*CYP2B6* 15582C→T*	0.04 (-0.13 to 0.21)	0.64	NA	NA	NA	NA
CYP2A6	*CYP2A6* -48A→C*	1.07 (0.80 to 5.17)	0.93	NA	NA	NA	NA

[†]The targeted SNPs (*CYP2B6* 516G→T, *CYP2A6* -48A→C, *CYP2B6* 983T→C, *CYP2B6* 15582C→T, *SLCO1B1* 521T→ C and *SLCO1B1*) included 47 patients and the rest 43 patients; *SNP of interest but did not meet criteria of p-value<0.01; Bonferroni corrected *P*-value 5.68 x10^{-05}; [§]*P*-value <0.05 accepted as significant for SNPs with a previously described association; NA= Not applicable; OR = odds ratio

Table S3. Genetic associations with detectable log_{10}-transformed plasma 8-OH-EFV/EFV, plasma 7-OH-EFV/EFV and CSF 8-OH-EFV/EFV concentrations in South African adults.[†]

Plasma 8-OH-EFV/EFV		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
CYP2B6	Composite *CYP2B6* 516/983	**-0.29 (-0.38 to -0.21)**	**3.7 x10^{-08}**	NA	NA	NA	NA
CYP2B6	*CYP2B6* 516G→T	**-0.27 (-0.39 to -0.15)**	**6.5 x10^{-05}**	NA	NA	NA	NA
CYP2B6	rs73557157	0.31 (0.17 to 0.45)	7.9 x10^{-05}	0.14 (-0.02 to 0.31)	0.09	0.06 (-0.09 to 0.21)	0.43
CYP2B6	rs60618302	0.28 (0.15 to 0.41)	1.1 x10^{-04}	0.13 (-0.02 to 0.28)	0.10	0.05 (-0.09 to 0.18)	0.51
ABCC4	rs8001444	-0.27 (-0.39 to -0.14)	1.6 x10^{-04}	-0.18 (-0.29 to -0.07)	2.2 x10^{-03}	-0.12 (-0.23 to -0.01)	3.2 x10^{-02}
CYP2B6	rs59243457	-0.25 (-0.37 to -0.13)	1.8 x10^{-04}	0.14 (-0.14 to 0.41)	0.33	0.06 (-0.18 to 0.30)	0.61
PKD2	rs2728108	-0.36 (-0.54 to -0.18)	3.1 x10^{-04}	-0.24 (-0.40 to -0.08)	5.6 x10^{-03}	-0.18 (-0.32 to -0.04)	1.7 x10^{-02}
SLCO2B1	rs151119066	-0.48 (-0.76 to -0.21)	1.3 x10^{-03}	-0.43 (-0.64 to -0.23)	1.7 x10^{-04}	-0.31 (-0.51 to -0.11)	5.1 x10^{-03}
ABCC4	rs9524896	-0.23 (-0.37 to -0.10)	1.3 x10^{-03}	-0.15 (-0.27 to -0.04)	1.3 x10^{-02}	-0.10 (-0.20 to 0.01)	0.07
SLCO2B1	rs114000664	-0.35 (-0.56 to -0.14)	1.9 x10^{-03}	-0.25 (-0.42 to -0.08)	5.6 x10^{-03}	-0.20 (-0.35 to -0.05)	1.2 x10^{-02}
ABCC4	rs1764417	-0.20 (-0.33 to -0.08)	3.1 x10^{-03}	-0.13 (-0.24 to -0.03)	1.9 x10^{-02}	-0.08 (-0.18 to 0.02)	0.13
SLCO2B1	rs2712788	-0.21 (-0.34 to -0.08)	3.4 x10^{-03}	-0.14 (-0.25 to -0.03)	1.9 x10^{-02}	-0.08 (-0.18 to 0.02)	0.14
ABCC4	rs114827818	-0.40 (-0.66 to -0.15)	3.4 x10^{-03}	-0.26 (-0.49 to -0.06)	1.4 x10^{-02}	-0.16 (-0.36 to 0.04)	0.13
SLCO2B1	rs143837090	-0.40 (-0.66 to -0.15)	3.5 x10^{-03}	-0.27 (-0.48 to -0.06)	1.5 x10^{-02}	-0.16 (-0.36 to 0.04)	0.13
ABCC4	rs58721524	-0.44 (-0.72 to -0.16)	4.1 x10^{-03}	-0.31 (-0.54 to -0.08)	1.1 x10^{-02}	-0.16 (-0.38 to 0.07)	0.19
ABCC4	rs6076964	-0.44 (-0.72 to -0.16)	4.1 x10^{-03}	-0.31 (-0.54 to -0.08)	1.1 x10^{-02}	-0.16 (-0.38 to 0.07)	0.19
CYP2B6	rs10401226	-0.19 (-0.32 to -0.07)	4.7 x10^{-03}	0.31 (0.09 to 0.52)	7.4 x10^{-03}	0.18 (-0.03 to 0.39)	0.10
CYP2B6	*CYP2B6* 983T→C	**-0.28 (-0.46 to -0.09)**	**5.5 x10^{-03}**	**-0.31 (-0.45 to -0.16)**	**1.8 x10^{-04}**	NA	NA
EGLN2	rs76268776	-0.38 (-0.64 to -0.13)	5.8 x10^{-03}	-0.31 (-0.51 to -0.11)	3.9 x10^{-03}	-0.26 (-0.43 to -0.09)	5.7 x10^{-03}
ABCC4	rs150301651	-0.30 (-0.50 to -0.09)	7.1 x10^{-03}	-0.22 (-0.37 to -0.06)	9.4 x10^{-03}	-0.15 (-0.30 to -0.004)	0.05
ABCG2	rs2725256	-0.19 (-0.32 to -0.05)	8.1 x10^{-03}	-0.12 (-0.23 to -0.01)	3.6 x10^{-02}	-0.08 (-0.18 to 0.02)	0.11
SLCO2B1	rs2510657	-0.18 (-0.32 to -0.05)	9.6 x10^{-03}	-0.11 (-0.22 to 0.004)	0.06	-0.04 (-0.15 to 0.07)	0.46

CYP2B6	*CYP2B6* 15582C→T*§	0.17 (-0.05 to 0.39)	0.14	0.04 (-0.14 to 0.23)	0.66	-0.06 (-0.22 to -0.11)	0.49
CYP2A6	*CYP2A6* -48A→C*	-0.17 (-0.40 to 0.07)	0.17	-0.15 (-0.34 to 0.05)	0.14	0.04 (-0.15 to 0.25)	0.64
Plasma 7-OH-EFV/EFV		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
CYP2A6	rs56164728	-0.50 (-0.72 to -0.27)	1.1 x10^{-04}	-0.49 (-0.73 to -0.25)	2.7 x10^{-04}	-0.47 (-0.71 to -0.23)	3.6 x10^{-04}
CYP2B6	**rs2279345**	**-0.28 (-0.42 to -0.15)**	**1.8 x10^{-04}**	**-0.29 (-0.44 to -0.14)**	**4.5 x10^{-04}**	**-0.28 (-0.43 to -0.12)**	**1.2 x10^{-03}**
CYP2A6	rs10853742	-0.28 (-0.42 to -0.15)	2.5 x10^{-04}	-0.28 (-0.42 to -0.13)	5.1 x10^{-04}	-0.31 (-0.44 to -0.17)	6.7 x10^{-05}
ABCB1	rs115780656	-0.30 (-0.48 to -0.12)	2.1 x10^{-03}	-0.29 (-0.47 to -0.11)	2.6 x10^{-03}	-0.27 (-0.45 to -0.10)	4.6 x10^{-03}
ABCC4	rs2993590	-0.31 (-0.50 to -0.12)	2.7 x10^{-03}	-0.29 (-0.49 to -0.10)	4.7 x10^{-03}	-0.30 (-0.48 to -0.11)	3.7 x10^{-03}
ABCC4	rs7318327	-0.20 (-0.33 to -0.07)	4.5 x10^{-03}	-0.19 (-0.32 to -0.05)	8.6 x10^{-03}	-0.19 (-0.32 to -0.06)	7.8 x10^{-03}
CYP2A6	rs11878604	-0.17 (-0.28 to -0.06)	4.6 x10^{-03}	-0.16 (-0.27 to -0.05)	7.8 x10^{-03}	-0.19 (-0.30 to -0.08)	1.6 x10^{-03}
ABCC4	rs60338761	-0.27 (-0.46 to -0.09)	6.8 x10^{-03}	-0.26 (-0.45 to -0.06)	1.4 x10^{-02}	-0.25 (-0.45 to -0.06)	1.5 x10^{-02}
CYP2A6	rs7248240	-0.29 (-0.50 to -0.08)	8.8 x10^{-03}	-0.27 (-0.49 to -0.06)	1.7 x10^{-02}	-0.26 (-0.47 to -0.05)	2.0 x10^{-02}
CYP2B6	Composite *CYP2B6* 516/983	0.11 (-0.00 to 0.23)	0.06	NA	NA	NA	NA
CYP2B6	*CYP2B6* 516G→T*§	0.09 (-0.06 to 0.23)	0.24	NA	NA	NA	NA
CYP2B6	*CYP2B6* 983T→C*§	0.14 (-0.05 to 0.34)	0.16	0.17 (-0.05 to 0.38)	0.14	NA	NA
CYP2B6	*CYP2B6* 15582C→T*§	-0.00 (-0.23 to 0.22)	0.99	0.03 (-0.22 to 0.28)	0.83	0.09 (-0.17 to 0.35)	0.50
CYP2A6	*CYP2A6* -48A→C*	-0.10 (-0.34 to 0.13)	0.38	-0.15 (-0.34 to 0.05)	0.14	0.04 (-0.15 to 0.25)	0.64
CSF 8-OH-EFV/EFV (n=16)		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Gene	Polymorphism	β(95%CI)	*P*-value	β(95%CI)	*P*-value	β(95%CI)	*P*-value
ABCC5	rs6762938	0.36 (0.16 to 0.57)	3.5 x10^{-03}	0.38 (0.09 to 0.20)	1.4 x10^{-03}	0.34 (0.16 to 0.52)	3.3 x10^{-03}
ABCC5	rs6807271	0.35 (0.15 to 0.56)	4.8 x10^{-03}	0.35 (0.16 to 0.55)	3.9 x10^{-03}	0.33 (0.16 to 0.50)	2.5 x10^{-03}
EGLN2	rs76268776	-1.02 (-1.62 to -0.41)	5.6 x10^{03}	-0.97 (-1.59 to -0.35)	9.1 x10^{-03}	-0.83 (-1.62 to -0.03)	0.06
CYP2B6	Composite *CYP2B6* 516/983	-0.29 (-0.56 to -0.02)	0.06	NA	NA	NA	NA
CYP2B6	*CYP2B6* 516G→T*§	-0.15 (-0.47 to 0.16)	0.36	NA	NA	NA	NA

CYP2B6	*CYP2B6* 983T→C[*§]	-0.40 (-0.86 to 0.06)	0.11	-0.48 (-0.94 to -0.03)	0.05	NA	NA
CYP2B6	*CYP2B6* 15582C→T[*§]	0.09 (-0.41 to 0.60)	0.73	-0.06 (-0.64 to 0.51)	0.83	-0.07 (-0.54 to 0.41)	0.79
CYP2A6	*CYP2A6* -48A→C[*]	0.14 (-0.35 to 0.64)	0.58	0.09 (-0.43 to 0.61)	0.73	**0.54 (0.05 to 1.03)**	**4.8 x10^{-02}**

[†]The targeted SNPs (*CYP2B6* 516G→T, *CYP2A6* -48A→C, *CYP2B6* 983T→C, *CYP2B6* 15582C→T, *SLCO1B1* 521T→ C and *SLCO1B1*) included 47 patients and the rest 43 patients; CSF = cerebrospinal fluid; EFV = efavirenz; CSF = cerebrospinal fluid; 08-OH-EFV = 8-hydroxy-efavirenz; 7-OH-EFV = 7-hydroxy-efavirenz; *SNP of interest but did not meet criteria of p-value<0.01; Bonferroni corrected *P*-value 5.68 x10^{-05}; [§]*P*-value <0.05 accepted as significant for SNPs with a previously described association; NA= Not applicable

Table S4. Genetic associations with detectable log_{10}-transformed plasma, CSF and CSF-to-plasma tenofovir concentrations in 43 South African adults.

Plasma tenofovir		Unadjusted analysis	
Gene	Polymorphism	β(95%CI)	*P*-value
ABCG2	rs2231159	-0.32 (-0.48 to -0.17)	2.2×10^{-04}
ABCC4	rs7982526	-0.61 (-0.93 to -0.29)	5.3×10^{-04}
ABCG2	rs45621036	-0.30 (-0.47 to -0.13)	1.1×10^{-03}
SLCO1A2	rs12809856	-0.24 (-0.39 to -0.10)	2.4×10^{-03}
ABCC5	rs35494670	-0.25 (-0.40 to -0.09)	3.2×10^{-03}
ABCG2	rs28440048	-0.31 (-0.52 to -0.11)	4.4×10^{-03}
ABCC5	rs6792482	-0.27 (-0.44 to -0.09)	5.8×10^{-03}
SLCO1A2	rs7968842	-0.21 (-0.36 to -0.07)	6.1×10^{-03}
ABCC5	rs56889675	-0.21 (-0.36 to -0.07)	7.4×10^{-03}
SLCO1A2	rs12296154	-0.19 (-0.33 to -0.06)	7.5×10^{-03}
CSF tenofovir		Unadjusted analysis	
Gene	Polymorphism	β(95%CI)	*P*-value
ABCC4	rs7982526	-0.58 (-0.85 to -0.30)	1.7×10^{-04}
ABCC5	rs35494670	-0.24 (-0.37 to -0.10)	1.4×10^{-03}
ABCG2	rs2231159	-0.23 (-0.38 to -0.08)	4.3×10^{-03}
ABCB1	rs28401796	-0.25 (-0.41 to -0.08)	5.2×10^{-03}
ABCB1	rs28381940	-0.25 (-0.41 to -0.08)	5.2×10^{-03}
SLCO1A2	rs4762700	-0.17 (-0.29 to -0.05)	9.6×10^{-03}
SLCO1A2	rs140377659	0.23 (0.06 to 0.40)	9.8×10^{-03}
CSF-to-plasma tenofovir		Unadjusted analysis	
Gene	Polymorphism	β(95%CI)	*P*-value

ABCB1	rs1989830	-0.12 (-0.19 to -0.05)	1.2 x10^{-03}
ABCB1	rs78551545	-0.28 (-0.43 to -0.12)	1.3 x10^{-03}
SLCO1A2	rs11535999	0.13 (0.05 to 0.21)	2.5 x10^{-03}
ABCG2	rs111917717	-0.28 (-0.46 to -0.11)	3.0 x10^{-03}
ABCG2	rs76462878	-0.22 (-0.36 to -0.08)	4.0 x10^{-03}
ABCB1	rs35572298	-0.18 (-0.30 to -0.06)	5.0 x10^{-03}
SLCO1A2	rs4149008	0.11 (0.03 to 0.18)	6.9 x10^{-03}
SLCO1A2	rs4149009	0.11 (0.03 to 0.19)	7.8 x10^{-03}
SLCO1A2	rs10841786	0.11 (0.03 to 0.19)	7.8 x10^{-03}
SLCO1A2	rs57472326	0.11 (0.03 to 0.19)	7.8 x10^{-03}
SLCO1A2	rs7968842	0.11 (0.03 to 0.19)	9.2 x10^{-03}

CSF = cerebrospinal fluid; Bonferroni corrected *P*-value 5.68 x10^{-05}

Table S5. Genetic associations with detectable log_{10}-transformed plasma, CSF and CSF-to emtricitabine concentrations in 39 South African adults.

Plasma emtricitabine		Unadjusted analysis	
Gene	Polymorphism	β(95%CI)	*P*-value
ABCC5	rs56889675	-0.26 (-0.40 to -0.12)	8.0 x10^{-04}
ABCC5	rs74763842	0.20 (0.07 to 0.33)	4.4 x10^{-03}
ABCC5	rs10470524	-0.25 (-0.42 to -0.08)	6.2 x10^{-03}
SLCO1A2	rs12296154	-0.20 (-0.34 to -0.07)	6.4 x10^{-03}
ABCC4	rs7982526	-0.54 (-0.91 to -0.16)	7.7 x10^{-03}
SLCO2B1	rs10793116	-0.42(-0.72 to -0.12)	8.8 x10^{-03}
CSF emtricitabine		Unadjusted analysis	
Gene	Polymorphism	β(95%CI)	*P*-value
ABCC5	rs56889675	-0.32 (-0.50 to -0.15)	7.2 x10^{-04}
ABCC4	rs7982526	-0.81 (-1.25 to -0.36)	9.9 x10^{-04}
SLCO1A2	rs4762700	-0.28 (-0.45 to -0.11)	2.3 x10^{-03}
ABCC5	rs11921035	-0.54 (-0.88 to -0.21)	3.2 x10^{-03}
SLCO1A2	rs12296154	-0.27 (-0.43 to -0.10)	3.8 x10^{-03}
ABCC4	rs2484983	-0.32 (-0.53 to -0.11)	4.5 x10^{-03}
ABCC4	rs60338761	-0.40 (-0.66 to -0.13)	5.7 x10^{-03}
ABCC4	rs4773884	-0.25 (-0.42 to -0.08)	6.6 x10^{-03}
ABCC5	rs11928606	-0.47 (-0.79 to -0.15)	6.9 x10^{-03}
ABCC5	rs10937161	-0.34 (-0.57 to -0.10)	7.5 x10^{-03}
ABCB1	rs28401796	-0.34 (-0.58 to -0.10)	8.1 x10^{-03}
ABCB1	rs28381940	-0.34 (-0.58 to -0.10)	8.1 x10^{-03}
CSF-to-plasma emtricitabine		Unadjusted analysis	

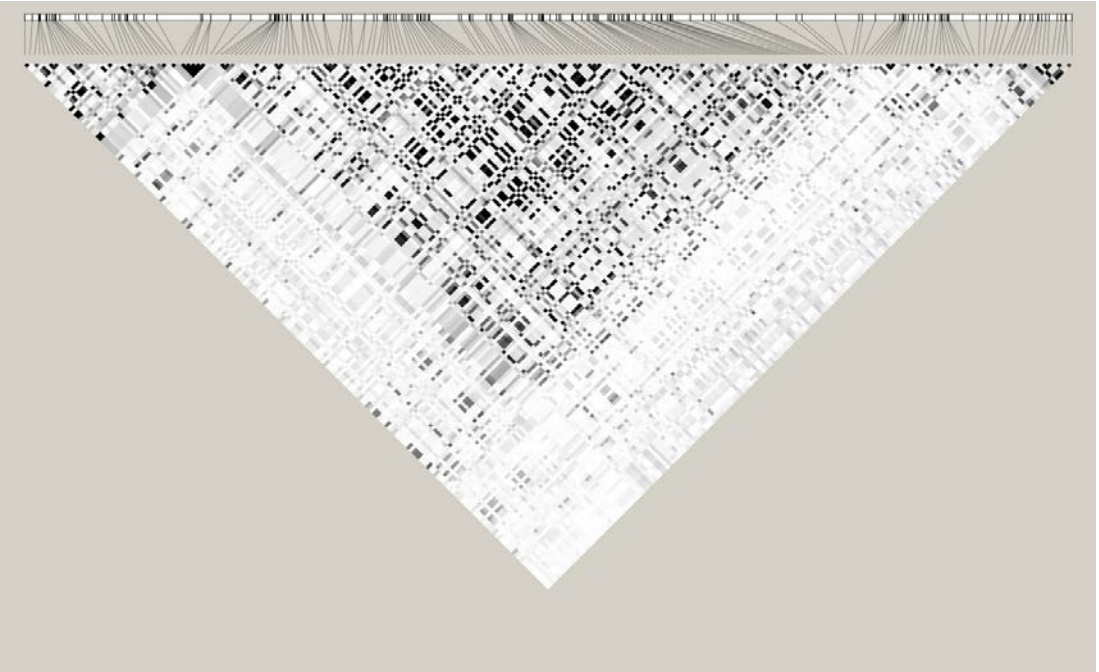
Gene	Polymorphism	β(95%CI)	*P*-value
ABCC5	rs11921035	-0.32 (-0.50 to -0.14)	1.4 x10^{-03}
SLCO1A2	rs4762700	-0.16 (-0.25 to -0.06)	2.0 x10^{-03}
ABCC5	rs11928606	-0.27 (-0.44 to -0.10)	4.4 x10^{-03}
ABBC4	rs7322318	0.13 (0.05 to 0.22)	5.3 x10^{-03}
ABCC4	rs9590228	0.13 (0.04 to 0.22)	6.5 x10^{-03}
ABCC4	rs4148428	-0.18 (-0.30 to -0.06)	7.4 x10^{-03}
ABCC5	rs116312201	0.19 (0.06 to 0.33)	9.0 x10^{-03}
ABCG2	rs1448784	-0.32 (-0.54 to -0.09)	9.3 x10^{-03}

CSF = cerebrospinal fluid; Bonferroni corrected *P*-value 5.68 x10^{-05}

Supplementary material: linkage disequilibrium plots

Figure S1. Linkage disequilibrium (LD) between polymorphisms in the *ABCC5* locus from chromosome 3 created in Haploview. Data from 43 participants are included. Figure **1(a)** includes all polymorphisms successfully genotyped that were not monomorphic in this cohort. Black denotes r^2=1, shades of grey, 0 < r^2 < 1, white r^2=0. Figure **1(b)** includes only polymorphisms with P < 0.01 for association with at least one concentration value in at least one unadjusted analyses. r^2 is shown to display LD.

(a)



(b)

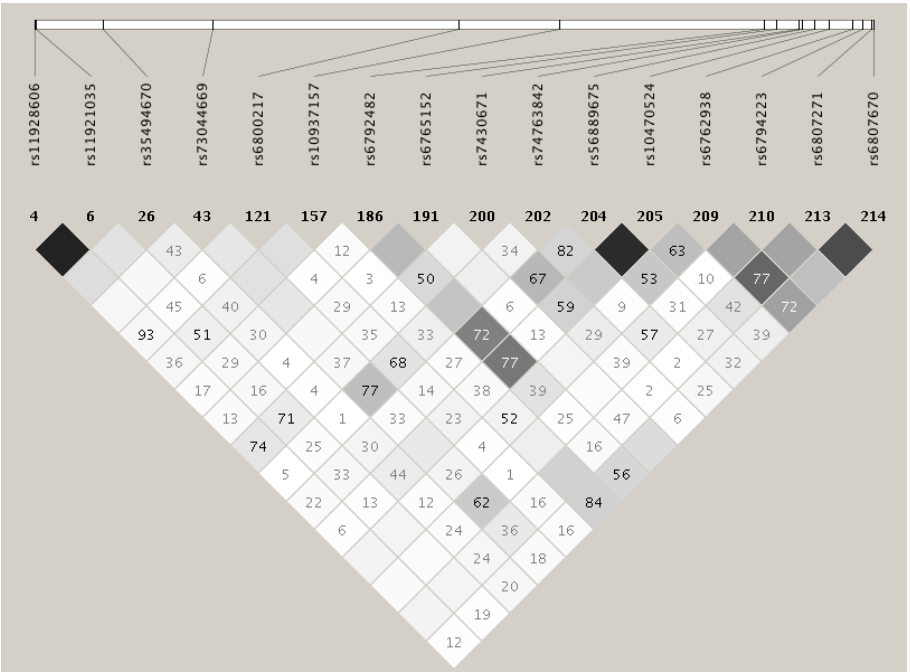
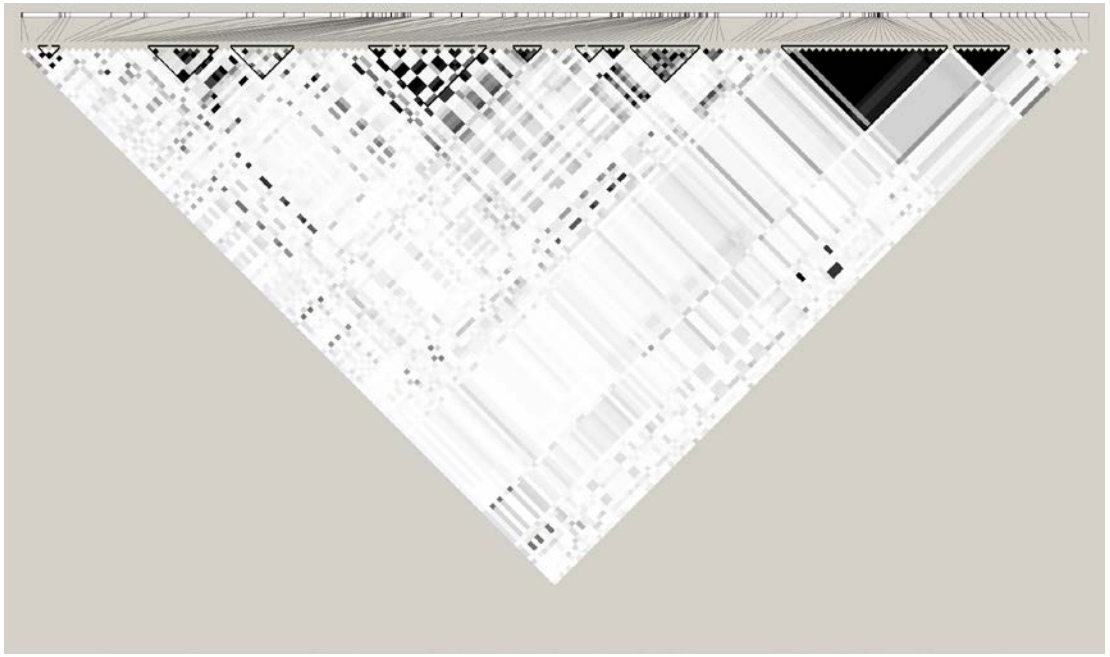


Figure S2. Linkage disequilibrium (LD) between polymorphisms in the *ABCG5* locus from chromosome 4 created in Haploview. Data from 43 participants are included. Figure **2(a)** includes all polymorphisms successfully genotyped that were not monomorphic in this cohort. Black denotes r^2=1, shades of grey, 0 < r^2 < 1, white r^2=0. Figure **2(b)** includes only polymorphisms with P < 0.01 for association with at least one concentration value in at least one unadjusted analyses. r^2 is shown to display LD.

(a)



(b)

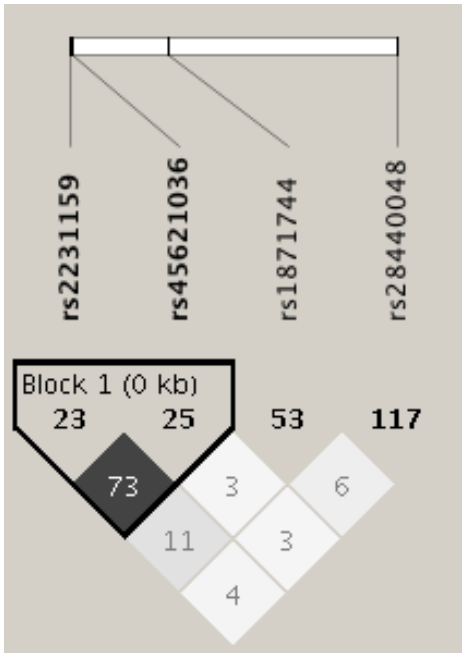
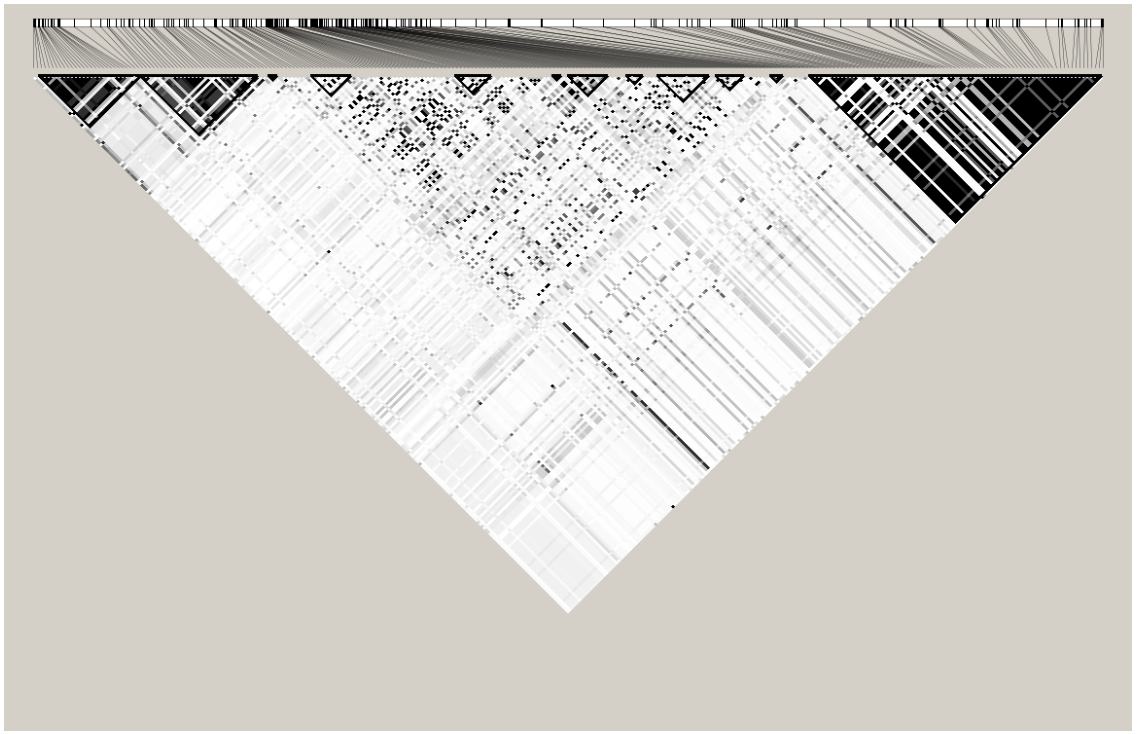


Figure S3. Linkage disequilibrium (LD) between polymorphisms in the *ABCB1* locus from chromosome 7 created in Haploview. Data from 43 participants are included. Figure **3(a)** includes all polymorphisms successfully genotyped that were not monomorphic in this cohort. Black denotes r^2=1, shades of grey, 0 < r^2 < 1, white r^2=0. Figure **3(b)** includes only polymorphisms with P < 0.01 for association with at least one concentration value in at least one unadjusted analyses. r^2 is shown to display LD.

(a)



(b)

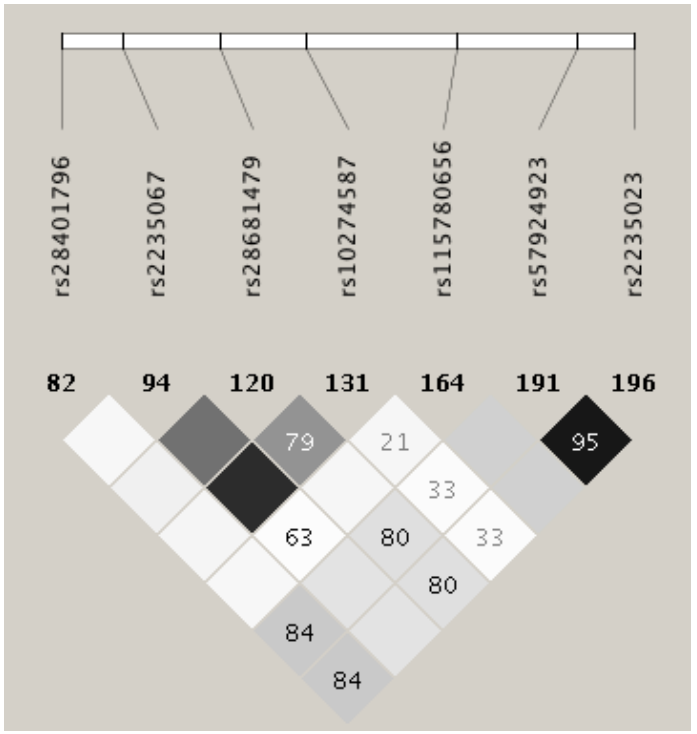
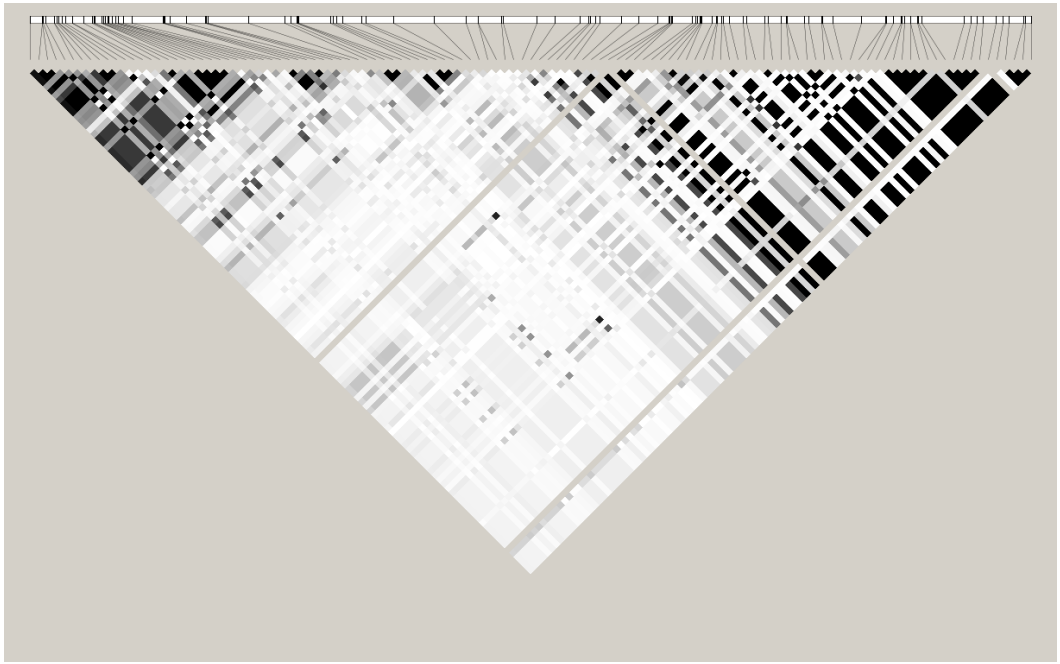


Figure S4. Linkage disequilibrium (LD) between polymorphisms in the *SLCO2B1* locus from chromosome 11 created in Haploview. Data from 43 participants are included. Figure **4(a)** includes all polymorphisms successfully genotyped that were not monomorphic in this cohort. Black denotes r^2=1, shades of grey, 0 < r^2 < 1, white r^2=0. Figure **4(b)** includes only polymorphisms with P < 0.01 for association with at least one concentration value in at least one unadjusted analyses. r^2 is shown to display LD.

(a)



(b)

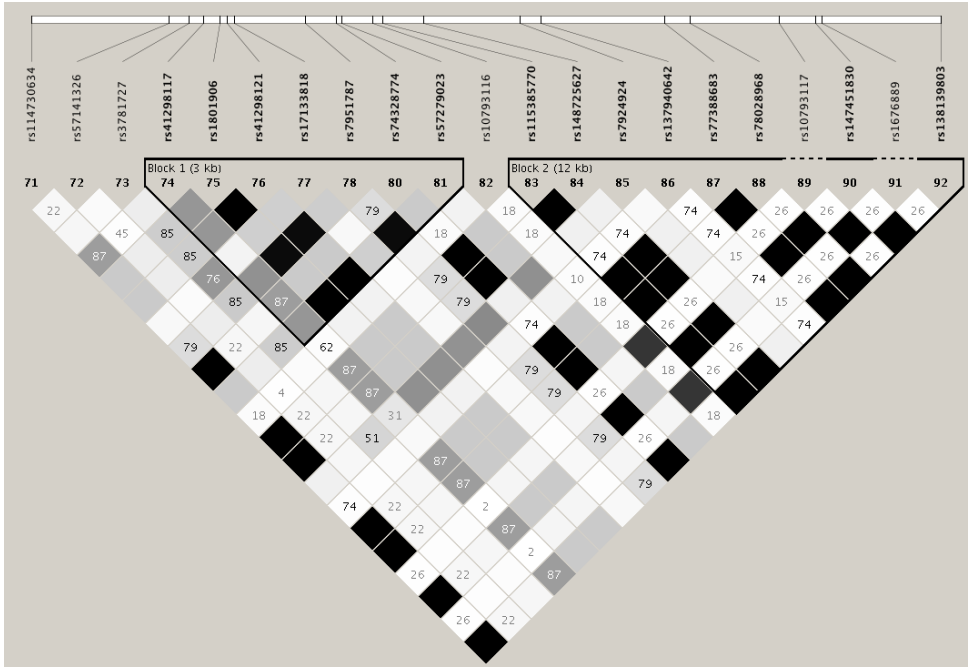
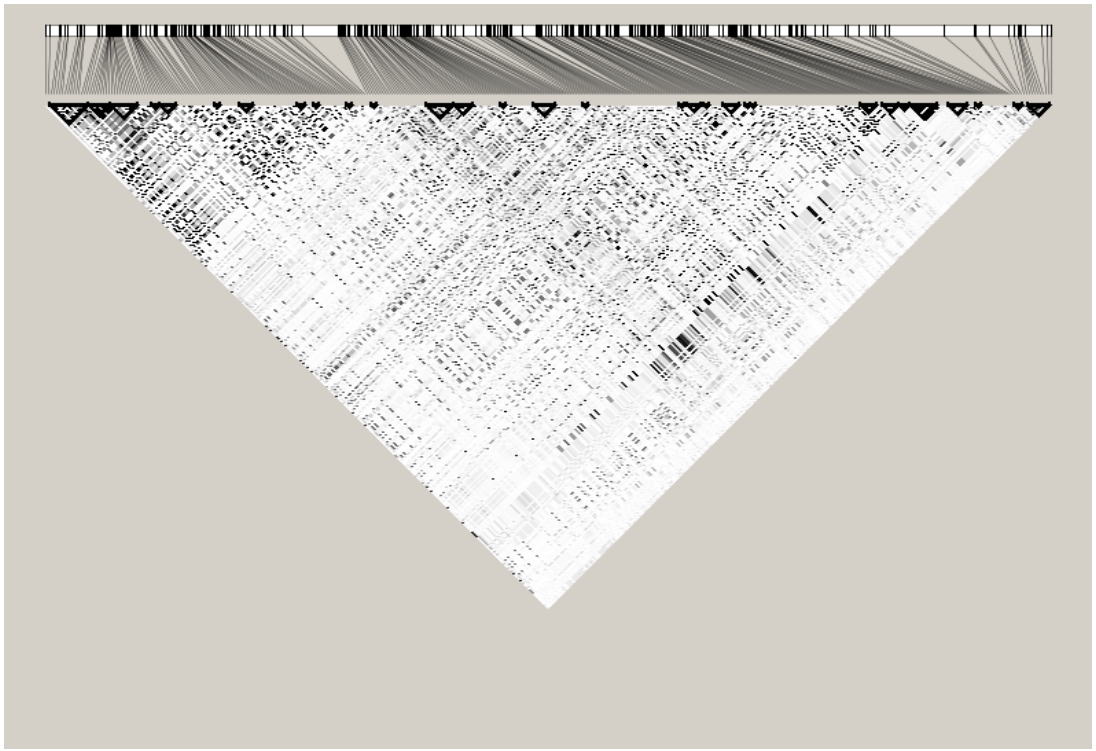


Figure S5. Linkage disequilibrium (LD) between polymorphisms in the *SLCO1A2* locus from chromosome 12 created in Haploview. Data from 43 participants are included. Figure **5(a)** includes all polymorphisms successfully genotyped that were not monomorphic in this cohort. Black denotes r^2=1, shades of grey, 0 < r^2 < 1, white r^2=0. Figure **5(b)** includes only polymorphisms with P < 0.01 for association with at least one concentration value in at least one unadjusted analyses. r^2 is shown to display LD.

(a)



(b)

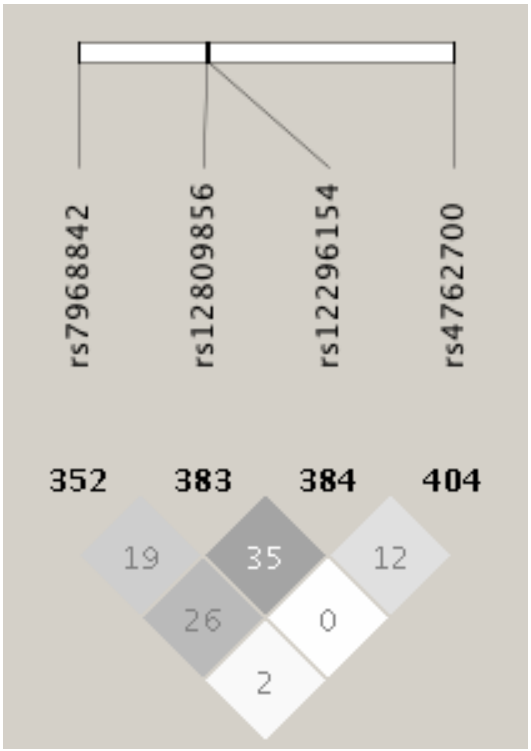
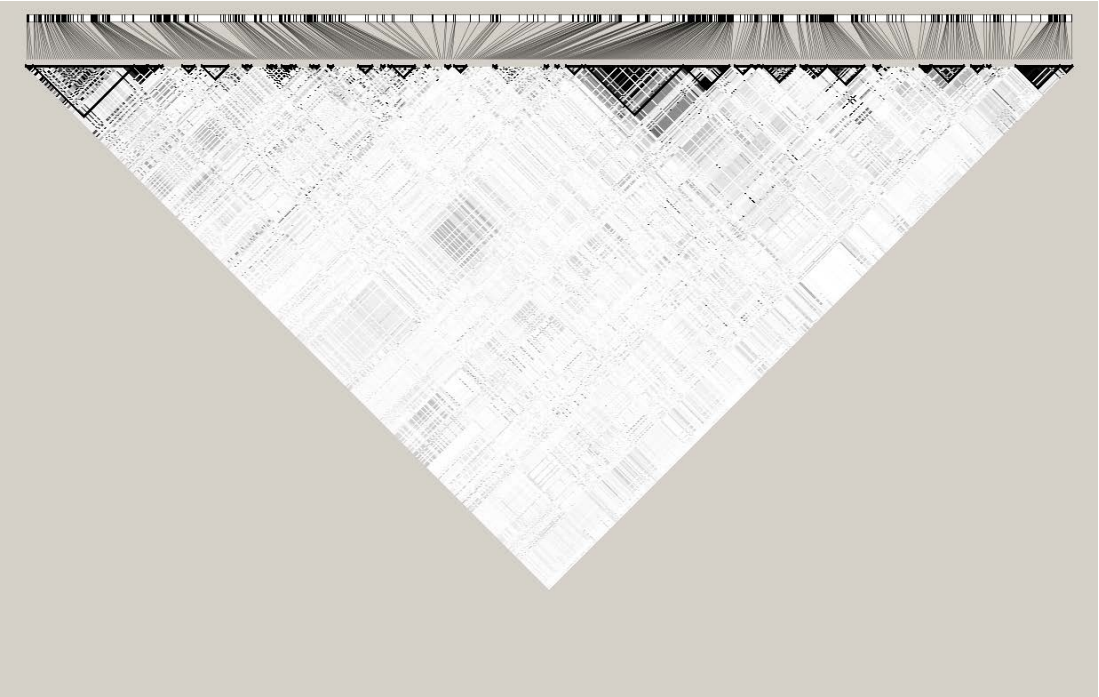


Figure S6. Linkage disequilibrium (LD) between polymorphisms in the *ABCC4* locus from chromosome 13 created in Haploview. Data from 43 participants are included. Figure **6(a)** includes all polymorphisms successfully genotyped that were not monomorphic in this cohort. Black denotes r^2=1, shades of grey, 0 < r^2 < 1, white r^2=0. Figure **6(b)** includes only polymorphisms with P < 0.01 for association with at least one concentration value in at least one unadjusted analyses. r^2 is shown to display LD.

(a)



(b)

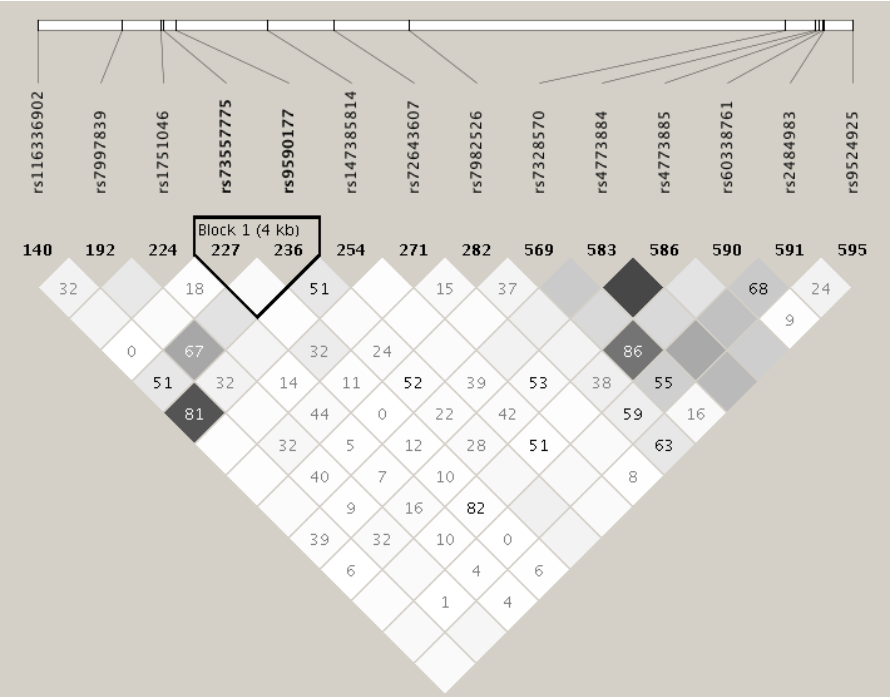
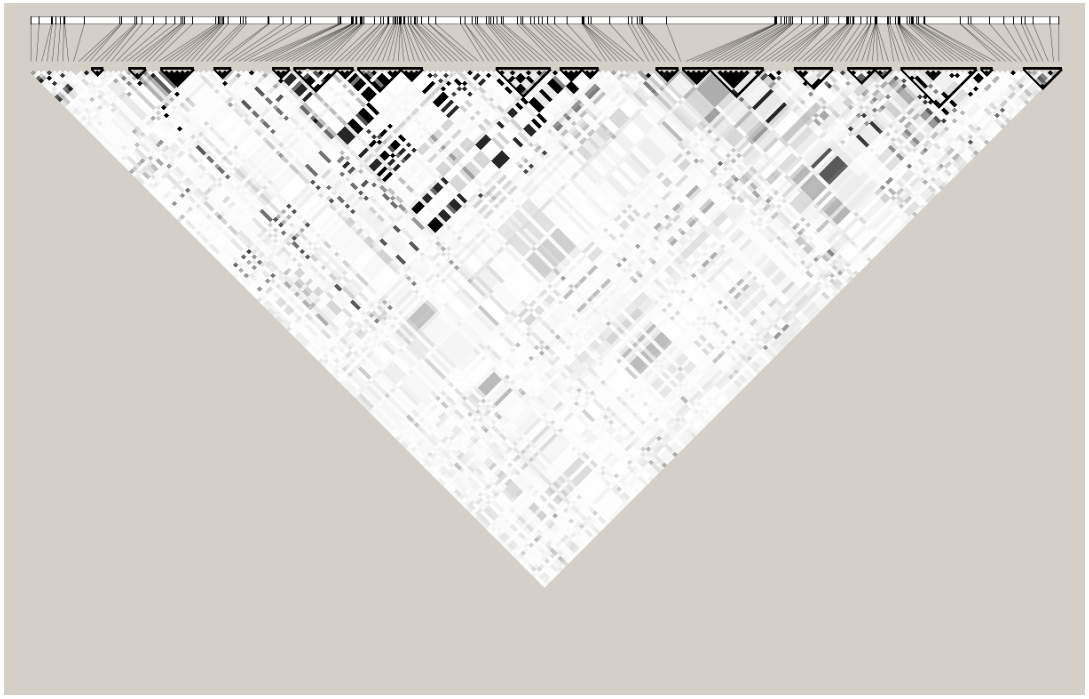
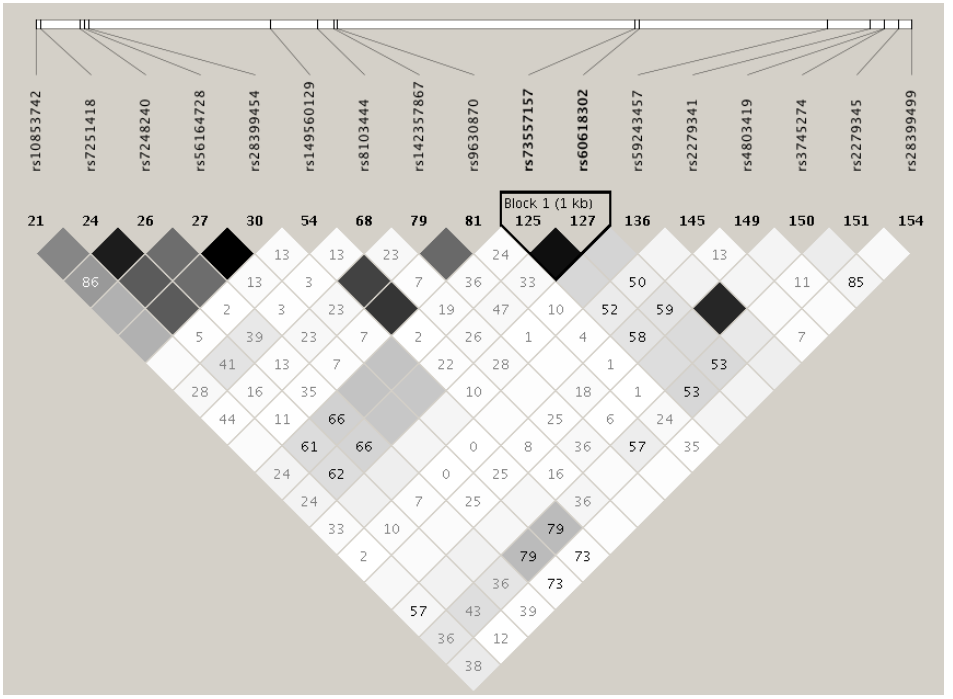


Figure S7. Linkage disequilibrium (LD) between polymorphisms in the *CYP2B6/A6* locus from chromosome 19 created in Haploview. Data from 43 participants are included. Figure **7(a)** includes all polymorphisms successfully genotyped that were not monomorphic in this cohort. Black denotes r^2=1, shades of grey, 0 < r^2 < 1, white r^2=0. Figure **7(b)** includes only polymorphisms with P < 0.01 for association with at least one concentration value in at least one unadjusted analyses as well as *CYP2B6* 983 T→C (rs28399499) and *CYP2B6* 15582 C→T (rs4803419). r^2 is shown to display LD.

(a)



(b)



CHAPTER 5

Moderate to severe HIV-associated neurocognitive impairment: a randomised placebo-controlled trial of lithium.

Clinical Trial/Experimental Study

Medicine®

OPEN

Moderate to severe HIV-associated neurocognitive impairment

A randomized placebo-controlled trial of lithium

Eric H. Decloedt, MBChB, FCCP, MMed[a,d,*], Carla Freeman, MBChB, FCPsych, MMed[b], Fleur Howells, BSc, BSc (Med)Hons, PhD[b], Martine Casson-Crook, MA[b], Maia Lesosky, BSc, MSc, PhD[c,g], Eleni Koutsilieri, DrMed Vet[f], Simon Lovestone, BM, MRCPsych, MPhil, PhD[e], Gary Maartens, MBChB, FCP, MMed, DTM&H[d], John A. Joska, MBChB, FCPsych, MMed, PhD[b]

Abstract

Background: HIV-associated neurocognitive disorder (HAND) remains highly prevalent despite effective anti-retroviral therapy (ART). A number of adjunctive pharmacotherapies for HAND have been studied with disappointing results, but preliminary data suggest that lithium may provide clinical benefit. In addition, the low cost of lithium would facilitate access in low- and middle-income countries which carry the greatest burden of HIV.

Methods: Our objective was to evaluate the 24-week efficacy and safety of lithium in patients with moderate to severe HAND. Our primary efficacy endpoint was the change in Global Deficit Score (GDS) from baseline to 24 weeks, whereas our secondary endpoint was the change in proton magnetic resonance spectroscopy (^{1}H-MRS) brain metabolite concentrations. We conducted a 24-week randomized placebo-controlled trial of lithium as adjunctive pharmacotherapy. We enrolled participants with moderate to severe HAND, on ART for at least 6 months, with suppressed viral loads and attending public sector primary care clinics in Cape Town, South Africa. We randomized 66 participants to lithium (n = 32) or placebo (n = 34). Lithium or placebo was dosed 12-hourly and titrated to achieve the maintenance target plasma concentration of 0.6 to 1.0 mmol/L. Sham lithium concentrations were generated for participants receiving placebo.

Results: Totally 61 participants completed the study (lithium arm = 30; placebo arm = 31). Participants at enrolment had a mean age of 40 years and a median CD4+ T-cell count of 500 cells/μL. The median change in GDS between baseline and week 24 for the lithium and placebo arms were –0.57 (95% confidence interval [CI] –0.77, –0.32) and –0.56 (–0.69, –0.34) respectively, with a mean difference of –0.054 (95% CI –0.26, 0.15); $P = 0.716$. The improvement remained similar when analyzed according to age, severity of impairment, CD4+ count, time on ART, and ART regimen. Standard ^{1}H-MRS metabolite concentrations were similar between the treatment arms. The study drug was well tolerated in both study arms. Six serious adverse events occurred, but none were considered related to the study drug.

Editor: Duane R. Hospenthal.

Statistical analysis: Dr Maia Lesosky, Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, University of Cape Town, South Africa.

Search terms: South Africa; Randomized controlled clinical trial; HIV neurocognitive impairment; HIV; Lithium; Placebo; Antiretroviral therapy

Authorship: ED—study concept and design, analysis and interpretation of data, drafting and revising the manuscript for content, acquisition of data, study supervision, and obtaining funds; CF—revising the manuscript for content, interpretation of data, acquisition of data, study supervision and coordination; FH—analysis and interpretation of data; MC-C—analysis and interpretation of data, revising the manuscript for content, acquisition of data and study coordination; ML—analysis and interpretation of data, statistical analysis; EK—study concept and design, revising the manuscript for content, and obtaining funds; SL—study concept and design, revising the manuscript for content and obtaining funds; GM—study concept and design, revising the manuscript for content, and obtaining funds; JJ—study concept and design, revising the manuscript for content, study supervision, and obtaining funds.

Disclosure: Norgine Pty (Ltd) unconditionally donated lithium carbonate and identical placebo, and had no input in any aspect of the study.

Funding: This study was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP Grant number SP.2011.41304.065/BMBF 01KA1306).

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

[a] Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, [b] Division of Neuropsychiatry, Department of Psychiatry and Mental Health, Faculty of Health Sciences, [c] Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, [d] Division of Clinical Pharmacology, Department of Medicine, Faculty of Health Sciences, University of Cape Town, South Africa, [e] Department of Psychiatry, University of Oxford, United Kingdom, [f] Institute of Virology and Immunobiology, University of Würzburg, Germany, [g] Department of Medicine, Faculty of Health Sciences, University of Cape Town, South Africa.

[*] Correspondence: Eric H. Decloedt, Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa (e-mail: ericdecloedt@sun.ac.za).

Medicine (2016) 95:46(e5401)

Received: 16 May 2016 / Received in final form: 14 September 2016 / Accepted: 22 October 2016

http://dx.doi.org/10.1097/MD.0000000000005401

Conclusion: Adjunctive lithium pharmacotherapy in patients on ART with HAND was well tolerated but had no additional benefit on neurocognitive impairment.

Abbreviations: ^{1}H-MRS = proton magnetic resonance spectroscopy, AIDS = acquired immune deficiency syndrome, ART = anti-retroviral therapy, CES-D = Center for Epidemiologic Studies Depression, Cho = choline, CI = confidence interval, Cr = creatine, DSMB = Data and safety monitoring board, eGFR = estimated glomerular filtration rate, FDR = false discovery rate, GDS = Global Deficit Score, Glx = glutamate with glutamine, GSK-3-β = glycogen synthase kinase-3-beta, HAND = HIV-associated neurocognitive disorder, mI = Myo-inositol, NAA = *N*-acetyl-aspartate, NAA+NAAG = *N*-acetyl-asparate with *N*-acetyl-aspartyl-glutamate, PACTR = Pan African Clinical Trials Registry, TETRAS = TRG Essential Tremor Rating Assessment Scale.

Keywords: antiretroviral therapy, HIV, HIV neurocognitive impairment, lithium, Placebo, randomized controlled clinical trial, South Africa

1. Introduction

HIV-associated neurocognitive disorder (HAND) remains highly prevalent despite effective antiretroviral therapy (ART).[1,2] The incidence of severe HAND has decreased, but with longer life expectancy and associated risk factors for cerebrovascular disease, the overall prevalence of HAND is projected to rise.[3,4] HAND is associated with high rates of morbidity and mortality.[1,5,6] Effective neuroprotective adjunctive pharmacotherapy for HAND has not yet been identified.

A number of adjunctive pharmacotherapies for HAND have been studied with disappointing results thus far.[7] Preliminary data suggest that lithium may provide clinical benefit as adjunctive pharmacotherapy. In 2 pilot studies, adjunctive lithium in HAND improved neurocognitive impairment in 1 study, whereas neuronal integrity on imaging improved in both studies.[8,9] However, these pilot studies were limited by both the lack of a comparator arm and the short duration of lithium treatment. Lithium has also been associated with an increase in gray matter volume on neuroimaging in other patient populations.[10] In addition, lithium has been associated with an improvement in neurocognitive impairment in patients with Alzheimer's disease.[11] Lithium has complex pharmacological effects but unequivocal is the inhibition of glycogen synthase kinase-3-beta (GSK-3-β), a serine-threonine protein kinase, that mediates neuronal function, cellular substrates for learning and memory, as well as neuronal apoptosis and inflammation signaling pathways.[12–14] In addition to the potential promise of lithium as an adjuvant from preliminary work, its low cost would facilitate access in low- and middle-income countries which carries the greatest burden of HIV.

We conducted a 24-week randomized placebo-controlled trial to study lithium as an adjunctive pharmacotherapy in patients with moderate to severe HAND.

2. Methods

Our primary efficacy endpoint was the change in the Global Deficit Score (GDS) from baseline to 24 week in the placebo arm compared to the lithium arm. Baseline was the screening period up to 4 weeks prior to enrolment (–4–0 weeks). During the screening period all investigations and assessments were performed and week 1 started when the participant was enrolled and study drug dispensed. GDS summarizes the neuropsychological test results of selected cognitive domains and adjusts for age, education, gender, and ethnicity.[15] The following domains and tests were included: attention (Mental Alternation Test, Digit Span, Paced Auditory Serial Addition Test), learning and memory (the Hopkins Verbal Learning Test), motor speed (Finger Tapping Dominant Hand, Finger Tapping Non-Dominant Hand, Grooved Pegboard Test Dominant Hand, Grooved Pegboard Test Non-Dominant Hand), psychomotor speed (Trail Making Test A, Color Trails Test 1, Digit Symbol-Coding), executive function (Color Trails Test 2, Stroop Color-Word Test, Wisconsin Card-Sorting Test), visual learning and memory (Rey Complex Figure), and verbal fluency (Animals and Fruit and Vegetables). We screened for symptoms of depression using the Center for Epidemiologic Studies Depression (CES-D) scale.[16] Our secondary endpoint was the change between baseline (–4–0 weeks) and week 23 in proton magnetic resonance spectroscopy (^{1}H-MRS, TE30, and TR2000 ms) brain metabolite concentrations of glutamate, glutamate with glutamine (Glx), myo-inositol (mI), *N*-acetyl-aspartate (NAA), *N*-acetyl-asparate with *N*-acetyl-aspartyl-glutamate (NAA+NAAG), choline (Cho) and creatine (Cr) in 3 brain areas (cortical: anterior cingulate cortex, white matter: left frontal white matter and deep brain structure: left thalamus). The primary safety endpoint was the severity and frequency of adverse events.

2.1. Study design and participants

Inclusion criteria were HIV-infected adults (≥18 and ≤70 years), established on ART for at least 6 months with a suppressed viral load (HIV PCR <400 copies/mL), cognitive impairment as defined by a GDS ≥ 0.5 attending public sector ART clinics in Cape Town, South Africa. Enrolled participants were mainly recruited from Nolungile Site C clinic in Khayelitsha and were followed up at the University of Cape Town Clinical Research Centre at Groote Schuur Hospital. Eligible participants gave written informed consent; female participants were not pregnant or breastfeeding and females of child-bearing potential committed to use of contraception. We required additional written informed consent from each participant's care giver as we anticipated that participants may vary in their ability to provide consent (participants may understand the need for ART and that they have impaired memory, but may not be able to recall all aspects of the study procedures and risks). Care givers had to accompany participants to each study visit. We excluded participants who received an investigational drug within 30 days, had evidence of an active acquired immune deficiency syndrome (AIDS)-defining opportunistic infection, had a history of drug or alcohol abuse within 3 months before screening, had a positive urine drug screen for drugs of abuse (amphetamine, benzodiazepine, cannabis, cocaine, opiate), had confirmed neurosyphilis or vitamin B12 deficiency, had imaging structural abnormalities, had a significant head injury or severe mental illness. We minimized the risk of lithium exposure by excluding participants with a QTc greater than 450 ms for males and 470 ms for females, confirmed epilepsy on chronic treatment, use of any medications that may predispose the participant to lithium toxicity, clinically significant hypo- or hyperthyroidism or hypercalcaemia or hypermagnesaemia, renal impairment as

Table 1

Study procedures.

Week	–4 to 0 Visit 1	–4 to 0 Visit 2	–4 to 0 Visit 3	1 Visit 4	2 Visit 5	4 Visit 6	8 Visit 7	12 Visit 8	16 Visit 9	20 Visit 10	23 Visit 11	24 Visit 12
Informed consent	x											
Medical history and physical exam	x											
Lithium plasma concentration				x	x	x	x	x	x	x		x
Hematology*	x											x
Chemistry†	x					x	x	x	x	x		x
HIV viral load	x											x
β-HCG	x											x
Drug screen‡	x											
Electrocardiography	x					x	x	x	x	x		x
CD4+ T-cell count	x											x
Neuropsychological battery	x											x
Tremor measurement	x			x	x	x	x	x	x	x		x
Magnetic resonance spectroscopy		x									x	
Investigational drug dispensing			x			x	x	x	x	x		
Adverse event monitoring			x	x	x	x	x	x	x	x	x	x

β-HCG = beta-human chorionic gonadotropin.

* Full blood count including differential.

† Screening: *Treponema palllidum* antibodies, vitamin B12, calcium, magnesium, thyroid stimulating hormone (T3 and T4 if TSH is abnormal), sodium, potassium, calcium, urea, and creatinine; Visit 6–12: sodium, potassium, calcium, urea, and creatinine; repeat thyroid-stimulating hormone (T3 and T4 if TSH is abnormal) at visit 12.

‡ Drug screen included cocaine, amphetamine, opioids, cannabis, and benzodiazepines.

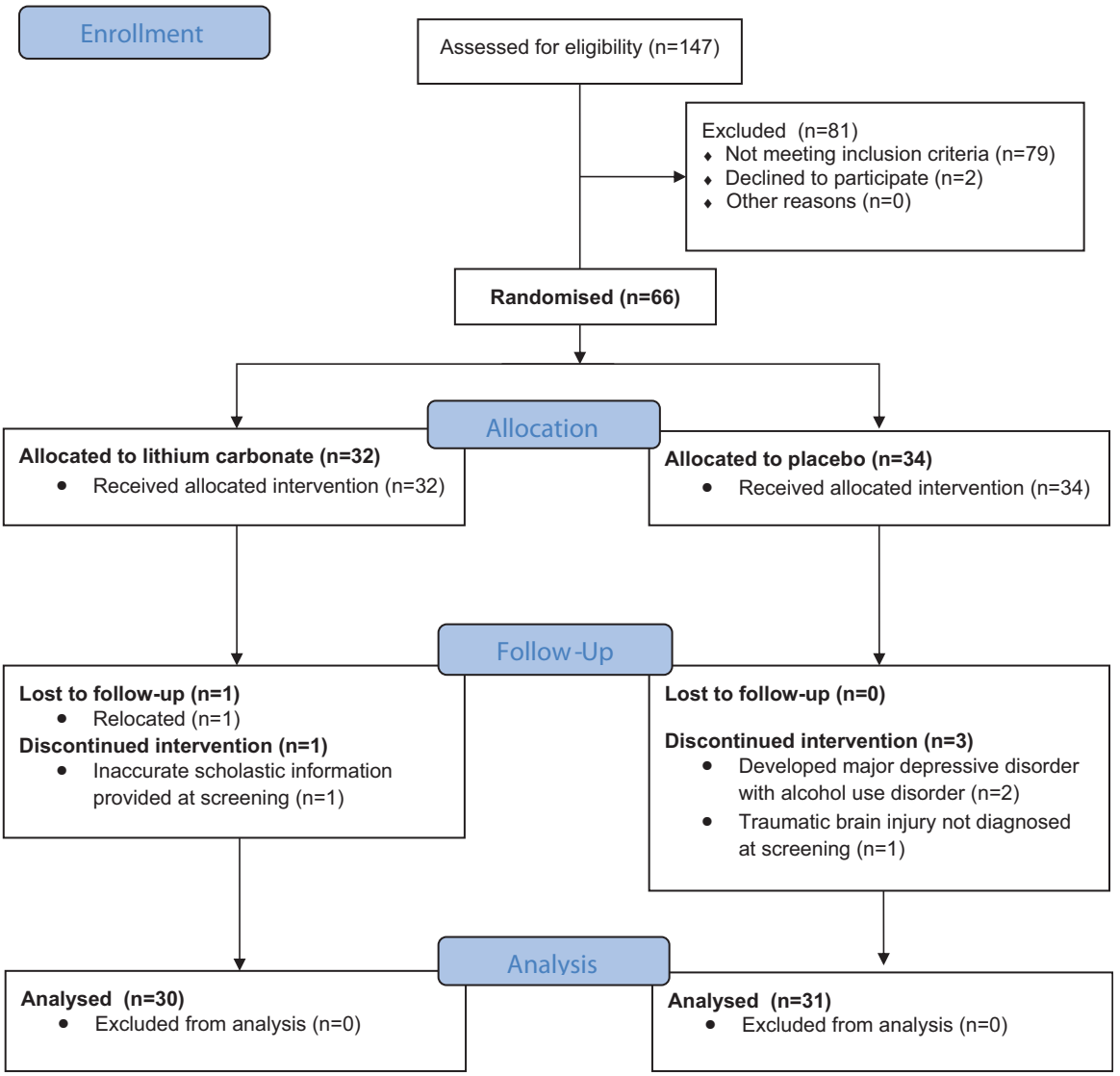


Figure 1. Diagram 1. Trial profile: eligibility, randomization, follow-up, and analysis.

defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min using the Cockroft and Gault formula and current diarrhea with dehydration.

2.2. Intervention

We dosed lithium carbonate 250 mg tablets (Camcolit, Norgine) and matching placebo (donated by Norgine). The investigational drugs were donated by Norgine who had no input into the study design, conduct, or analysis. Lithium was titrated to achieve the maintenance target plasma concentration of lithium in patients with bipolar mood disorder of between 0.6 and 1.0 mmol/L. Sham lithium concentrations were generated for participants receiving placebo (details in Section 2.4.)

2.3. Ethics and study oversight

The study was approved by the human research ethics committees of the University of Cape Town (071/2013) and Stellenbosch University (M13/07/027). The study was registered on the Pan African Clinical Trials Registry (PACTR) with the identifier number PACTR201310000635418. An independent data and safety monitoring board (DSMB) oversaw trial safety, whereas the trial steering committee mainly monitored progress of the trial.

2.4. Randomization, treatment concealment, and blinding

Participants in each cohort were randomized to placebo or the lithium carbonate prior to the start of the study using block randomization of 4, 6, or 8 which were subject to the overall constraint of adding to the total sample size. Once an enrolment number was assigned by the investigators, the study pharmacist dispensed treatment according to the randomization list. The statistician compiled the randomization list prior to study start. The randomization list was stored in a secure place with access limited to the statistician and pharmacist ensuring that the investigators and participants remained blinded throughout the study. Plasma concentrations were measured in both the lithium and placebo arms by the laboratory. The laboratory remained blinded and reported placebo concentrations as lower than level of detection. The laboratory forwarded the concentration results only to the study statistician. The study statistician generated sham lithium concentrations for the placebo patients and forwarded blinded concentrations (measured for lithium arm and simulated for placebo arm) to a coinvestigator who had no direct participant contact. This coinvestigator also received the adverse event logs, and in conjunction with the blinded concentrations, made dose-adjustment recommendations which were forwarded to the treating investigators. Only the study statistician was unblinded to arm allocation throughout this process. The sham lithium values were generated based on a random sampling from a distribution that was parameterized with the true measured lithium concentrations in the treatment arm, and with some additional rejection sampling to ensure the sham lithium values did not fall outside of feasible ranges.

2.5. Adverse events and safety investigations

We reviewed participants weekly for adverse events for the first month followed by 4 weekly visits for adverse events and adherence. Adherence was measured using pill counts and self-report diary cards. Suspected poor adherence was flagged by the study pharmacist when a >25% discrepancy in doses taken and the pill count was noted. Participants who were noted as potentially being poorly adherent were intensively counseled by the investigators and greater emphasis was placed on evaluating adherence at subsequent visits. Participants with clinically significant adverse events were reviewed more frequently as needed. At screening (–4 to 0 weeks) and week 24 we measured full blood count and differential, *Treponema palllidum* antibodies (screening only), vitamin B12 levels (screening only), chemistry (calcium, magnesium, thyroid function, sodium, potassium, calcium, urea, and creatinine), viral load, CD4+ count, urine screen for amphetamines, benzodiazepine, cannabis, cocaine, and opiate abuse (screening only) and β-HCG. At other visits (week 4, 8, 12, 16, 20), we measured lithium concentrations (actual and sham) and chemistry (sodium, potassium, calcium, urea and creatinine). Other safety investigations included electrocardiogram (screening, week 4, 8, 12, 16, 20, 24) and TRG Essential Tremor Rating Assessment Scale (TETRAS) (screening, week 1, 2, 4, 8, 12, 16, 20, 24). Neuroimaging was performed at baseline and week 23 (Table 1).

Table 2

Baseline characteristics.

Baseline characteristic	Lithium (n = 32)	Placebo (n = 34)	*P*
Gender			
Male	n = 6 (18%)	n = 2 (6%)	0.149[‖]
Female	n = 28 (82%)	n = 30 (94%)	
Age[*]	39.34 ± 8.07 y	40.59 ± 8.54 y	0.545[‡]
CD4+ T-cell count[†] cells/mm^3	502 (394–648)	498 (384–651)	0.788[§]
Months on ART[†]	51 (23–74.5)	40 (25–71)	0.640[§]
ART regimen			
NNRTI-based	n = 26 (81%)	n = 30 (88%)	0.327[‖]
PI-based	n = 6 (19%)	n = 4 (12%)	
Neurocognitive impairment			
GDS overall[†]	1.08 (0.83–1.44)	1.11 (0.82–1.53)	0.793[§]
GDS ≥ 1	n = 20 (62.5%)	n = 20 (58.8%)	0.479[‖]
GDS < 1	n = 12 (37.5%)	n = 14 (41.2%)	
Neuromedical assessment			
No disease	n = 18 (56%)	n = 24 (71%)	0.170[‖]
Mild-moderate disease	n = 14 (44%)	n = 10 (29%)	
Severe disease	n = 0	n = 0	
Years education			
≥ 10	n = 18 (56%)	n = 18 (53%)	0.491[‖]
< 10	n = 14 (44%)	n = 16 (47%)	
Employment status			
Employed[¶]	n = 8 (25%)	n = 13 (38%)	0.187[‖]
Unemployed	n = 24 (75%)	n = 21 (62%)	
Depression score			
CES-D	9 (4–17)	8 (3–14)	0.672[§]

ART = anti-retroviral therapy, CES-D = Center for Epidemiologic Studies Depression scale, GDS = Global Deficit Score.
[*] Mean and standard deviation.
[†] Median and interquartile range.
[‡] *t*-test (2 samples).
[§] Wilcoxon sum rank.
[‖] Fisher's exact test.
[¶] Full-time or part-time work;

2.6. Statistical methods

We calculated our sample size to detect an absolute value change in GDS of 0.25 and required 49 participants per arm for 90% power at alpha 0.05. We aimed to enroll 54 participants in each arm to account for a 10% loss to follow-up or withdrawal. Previous research has shown that ART alone improved the GDS

Table 3

Intent to treat analysis of neuropsychological changes.

Neuropsychological domain	Baseline *Lithium*	Baseline *Placebo*	*P*	Week 24 *Lithium*	Week 24 *Placebo*	*P*
Attention						
Digit Span	6.06 ± 1.27*	6.24 ± 1.23*	0.577‡	6.34 ± 1.50*	6.62 ± 1.35*	0.491‡
Paced Auditory Serial Addition Test	15.0 (12.0–19.5)†	16.00 (13.0–22.0)†	0.908§	17.50 (11.5–23.0)†	17.0 (14.0–24.0)†	0.792§
Learning and memory						
Hopkins Verbal Learning Test (recall)	5.0 (5.0–6.0)†	6.0 (4.0–7.0)†	0.319§	7.0 (7.0–8.0)†	8.0 (5.0–9.0)†	0.773§
Motor speed						
Finger Tapping nondominant hand	7.58 (6.70–8.73)†	7.83 (6.84–8.64)†	0.868§	6.78 (6.30–7.88)†	7.31 (6.28–8.16)†	0.256§
Grooved Pegboard Test non dominant hand	96.60 (79.96–123.04)†	101.43 (84.13–118.29)†	0.635§	80.36 (75.16–96.79)†	89.03 (80.94–103.58)†	0.0704§
Psychomotor speed						
Trail Making Test A	59.54 (46.46–88.58)†	66.62 (51.54–83.7)†	0.386§	47.05 (36.71–55.63)†	50.92 (37.7–65.67)†	0.218§
Color Trails Test 1	58.84 (50.58–82.15)†	78.07 (58.71–95.44)†	0.041§	64.23 (47.39–76.13)†	69.82 (61.05–84.86)†	0.093§
Digit Symbol-Coding	28.84 ± 10.55*	27.79 ± 9.22*	0.668‡	29.24 ± 11.28*	28.97 ± 10.36*	0.921‡
Executive function						
Color Trails Test 2	154.82 ± 45.95*	173.98 ± 41.04*	0.078‡	143.33 (111.52–169.12)†	146.44 (122.49–163.12)†	0.793§
Stroop Color-Word test	24.19 ± 8.25*	23.88 ± 8.53*	0.883‡	26.84 ± 9.17*	26.59 ± 8.55*	0.907‡
Wisconsin Card-Sorting Test	41.0 (31.0–87.5)†	42.0 (33.0–57.0)†	0.797§	41.0 (29.5–59.5)†	38.0 (32.0–45.0)†	0.542§
Visual learning and memory						
Rey Complex Figure (copy)	21.70 ± 7.87*	21.91 ± 8.0*	0.915‡	21.70 ± 7.87*	21.91 ± 8.00*	0.915‡
Rey Complex Figure (3 min)	10.44 ± 4.59*	10.56 ± 3.89*	0.908‡	10.44 ± 4.59*	10.56 ± 3.89*	0.908‡
Verbal fluency						
Animals	13.72 ± 2.96*	13.71 ± 3.75*	0.988‡	14.06 ± 2.66*	14.29 ± 2.93*	0.738‡
Fruit and vegetables	14.06 ± 3.05*	12.88 ± 2.88*	0.111‡	13.81 ± 3.18*	13.38 ± 3.24*	0.588‡
Depression score						
CES-D	9 (4–17)†	8 (3–14)†	0.672§	3 (0–8)†	4 (0–7)†	0.643§
Summary score						
Global Deficit Score	1.08 (0.83–1.44)†	1.11 (0.82–1.53)†	0.793§	0.73 (0.35–0.92)	0.74 (0.44–1.12)	0.329§

CES-D = Center for Epidemiologic Studies Depression scale.
* Mean and standard deviation.
† Median and interquartile range.
‡ *t*-test (2 samples).
§ Wilcoxon sum rank.

by a mean of 0.13 and 0.6 in patients with a GDS in the mild to moderate (>0.25 to <0.75) and severe (>0.75) ranges, respectively.[8,17] Twelve week adjunctive lithium therapy in patients stable on ART improved the GDS by 0.3 and we opted to detect a more conservative GDS difference of 0.25 with a standard deviation of 0.375, which was calculated using the range in the published studies divided by 4.[8,17] We conducted an intention-to-treat and per protocol analysis for the primary endpoint. For the intention-to-treat analysis, we carried over the last data points when the week 24 endpoints were missing, example for missing GDS at week 24 we used GDS at enrolment. For the per protocol analysis, we included only participants who completed the treatment originally allocated. We assessed the normality of the data visually and using the Shapiro–Wilk test. We compared baseline and week 24 values of continuous variables with paired *t*-tests or Wilcoxon sum rank depending on the distribution. Normally distributed data were described using the mean and standard deviation, whereas non-normally distributed data were described using median and interquartile ranges. We applied correction for the false discovery rate (FDR) by the method of Benjamin & Hochberg to comparisons. We report raw *P* values throughout and note any *P* values that lose or gain statistical significance after correction.

3. Results

We enrolled our first participant in December 2013 and had our last study visit in June 2015. Due to slow accrual we were unable to enroll our original calculated sample size and randomized 66 participants to lithium (n = 34) or placebo (n = 32), whereas 61 participants completed the study (lithium arm = 30; placebo arm = 31) (diagram 1). All participants were black Africans, first language Xhosa. Baseline characteristics were similar between the 2 groups with the majority of participants presenting with severe neurocognitive impairment with GDS of ≥ 1 (Table 2).

Suspected poor adherence was similar in the placebo and lithium arms. We recorded 47 poor adherence episodes of which 23 episodes occurred in 16 lithium arm participants and 24 episodes occurred in 17 placebo arm participants. In the 16 lithium arm participants: 10 participants had 1 poor adherence episode, 5 participants had 2 poor adherence episodes, and 1 participant had 3 poor adherence episodes. In the 17 placebo arm participants: 12 participants had 1 poor adherence episode, 3 participants had 2 poor adherence episodes, and 2 participants had 3 poor adherence episodes. The majority of poor adherence episodes occurred within the first 8 weeks of the study (57%). Week 24 viral loads were not predictive of poor adherence as the 2 participants with slightly raised viral loads at the end of the study (highest value 585 copies per mL) were not identified with poor adherence. Both participants were allocated to the lithium arms.

The improvement in GDS was not different between the treatment arms in both the intent-to-treat and the per protocol analysis (Table 3, supplemental file table 1, http://links.lww.com/MD/B409, diagram 2 (A) (B)). The median change in GDS scores between baseline and week 24 for the lithium and placebo arms were –0.57 (95% CI –0.77, –0.32) and –0.56 (–0.69, –0.34) respectively, with a mean difference of –0.054 (–0.26, 0.15);

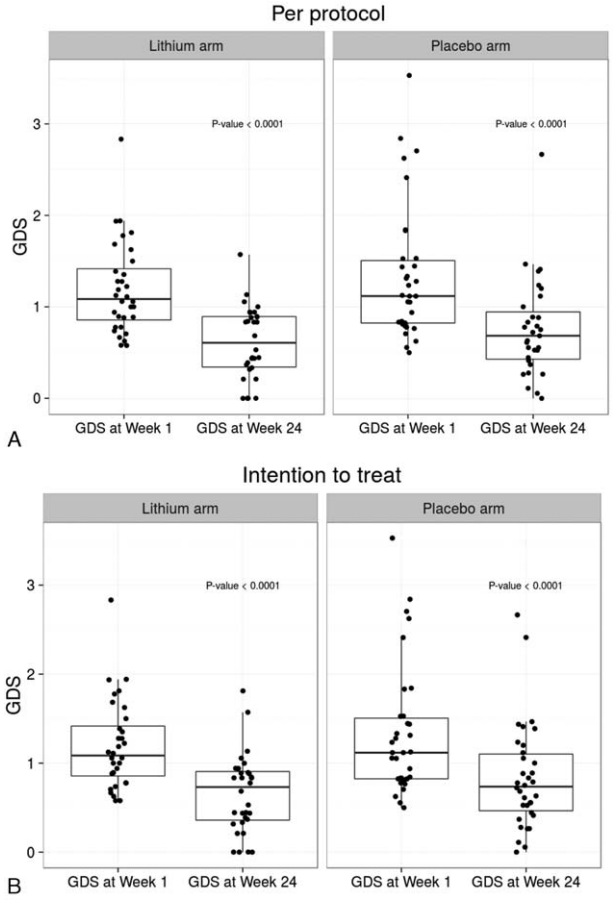


Figure 2. Diagram 2. Box-and-whisker plots of (GDS) at week 1 and week 24 analyzed (A) per protocol analysis and (B) intention to treat analysis. GDS = Global Deficit Score.

$P=0.716$. The improvement remained similar when analysed according to age, severity of impairment, CD4+ count, time on ART, and ART regimen. ^{1}H-MRS metabolite concentrations (supplemental file table 2, http://links.lww.com/MD/B409) were also not different between the treatment arms. However, the ^{1}H-MRS metabolite concentrations could not be measured for all participants due to intermittent periods of technical downtime of the MRI scanner. The study drug was well tolerated with no statistically significant difference ($P=0.413$) in total adverse events between the 2 study arms (Table 4). Six serious adverse events occurred but none were considered related to the study drug (supplemental file table 3, http://links.lww.com/MD/B409).

Table 4

Selected adverse events considered relevant to lithium therapy.

Adverse events	Severity	Lithium (n = 32)	Placebo (n = 34)
Cardiac disorders			
First degree heart block	Mild	1 (2.9%)	1 (3.1%)
Bradycardia	Mild	2 (5.9%)	1 (3.1%)
Tachycardia	Mild	0	1 (3.1%)
QTc prolongation	Mild	11 (32.4%)	14 (43.8%)
	Moderate	1 (2.9%)	0
ST-elevation	Mild	0	1 (3.1%)
T-wave changes	Mild	2 (5.9%)	0
Endocrine disorders			
Hypothyroidism	Mild	2 (5.9%)	0
Weight gain	Mild	1 (2.9%)	0
Symptoms of nephrogenic diabetes	Mild	1 (2.9%)	0
	Moderate	2 (5.9%)	0
Gastrointestinal disorders			
Abdominal cramps	Mild	2 (5.9%)	0
Constipation	Mild	0	1 (3.1%)
Diarrhoea	Mild	6 (17.6%)	7 (21.9%)
	Moderate	2 (5.9%)	1 (3.1%)
Dyspepsia	Mild	0	1 (3.1%)
Gastroenteritis	Mild	0	1 (3.1%)
Increased stool frequency	Mild	0	1 (3.1%)
Loose stool	Mild	0	2 (6.3%)
Investigations			
Hyperkalaemia	Mild	1 (2.9%)	1 (3.1%)
	Moderate	1 (2.9%)	0
Hypermagnesemia	Mild	1 (2.9%)	0
Hypomagnesemia	Mild	1 (2.9%)	0
Hypocalcaemia	Mild	2 (5.9%)	3 (9.4%)
Low vitamin B12	Mild	1 (2.9%)	0
Raise in viral load	Mild	2 (5.9%)	0
Nervous system disorders			
Dizziness	Mild	6 (17.6%)	4 (12.5%)
Headache	Mild	4 (11.8%)	12 (37.5%)
	Moderate	0	1 (3.1%)
Upper limb tremor	Mild	26 (76.5%)	26 (81.3%)
Lower limb tremor	Moderate	1 (2.9%)	0
Psychiatric disorders			
Daytime somnolence	Mild	2 (5.9%)	0
Insomnia	Mild	1 (2.9%)	0
Major depressive disorder with comorbid alcohol use	Severe	0	2 (6.3%)
Renal disorders			
Decrease in estimated glomerular filtration rate	Mild	1 (2.9%)	2 (6.3%)
Total		81 (49.4%)	83 (50.6%)

4. Discussion

Our study is the first to test adjunctive lithium therapy in patients with HAND in a randomized double blind controlled trial for a period of 6 months. We found that adjunctive lithium in patients with HAND was well tolerated but had no benefit on neurocognitive impairment compared with placebo when assessing neuropsychological test performance and ^{1}H-MRS metabolite concentrations. Neurocognitive impairment improved similarly in both the lithium and placebo arm.

Lithium has demonstrated neuroprotection with an increase in gray matter volume in various patient populations.[10,13] However, controlled clinical data demonstrating neuroprotection with clinical endpoints were lacking. The improvement in GDS we observed in the lithium arm is similar to the improvement noted by Letendre et al[8] (median improvement 0.29 while we found a median improvement of 0.47) in an open-label 12-week lithium study in patients with HAND. The similar improvement we observed in the placebo arm highlights the importance of a comparator arm. There are a number of potential explanations for our findings that lithium was no better than placebo. First, the placebo effect is a well-described response accompanied by psychobiological changes in the brain.[18] Clinicians are held in high regard and could have biased our participants' expectations and response.[19] Second, participants may have become more familiar with the neuropsychological assessments leading to a practice effect. We deliberately scheduled the neuropsychological assessments 6 months apart

to limit a potential practice effect, but cannot completely exclude some practice effect. In addition, no participant underwent a neuropsychological assessment prior to enrolment into this study. Third, we assessed endpoints only twice 6 months apart which prevents a longitudinal description of natural disease progression, placebo response, and lithium effect. The trajectory of natural disease, placebo, and lithium would be best described in longer term studies where quantitative modeling is applied.[20] The possibility exists that the placebo response may be temporary. Fourth, cognitive assessment is influenced by HIV infection, physical -, psychiatric -, and social comorbidity.[21] We monitored HIV -, physical- and psychiatric comorbidities and did not detect an improvement, but it is plausible that we missed social comorbidity improvement explained by trial participation. Lastly, it is possible that only patients with certain covariates or characteristics (such as depression comorbidity) may respond significantly better to lithium compared with placebo. Recently a genome-wide association between lithium response and common genetic variants on chromosome 21 has been identified in patients with bipolar disorder.[22]

Our study has a number of differences when compared with the open-label pilot studies of adjunctive lithium in HAND: longer study duration, randomized double-blind placebo-controlled design, lithium, and placebo dose adjusted using therapeutic drug monitoring with a target range used in the treatment of bipolar mood disorder and mostly African female participants.[8,9] The Letendre et al[8] study found that lithium improved the GDS from impaired to normal after 12 weeks in 8 participants, whereas Schiffitto et al found no neurocognitive improvement after 10 weeks in 13 participants, but found a decrease in glutamate with glutamine (Glx) metabolites in the frontal gray matter.[8,9] However, both studies were uncontrolled.

Our study has a number of limitations. First, our findings are limited by the fact that we were unable to enroll our original calculated sample due to slow accrual. However, an increase in sample size is unlikely to change our findings as an interim review by the DSMB determined that a sample size of 65 using the same assumptions as the original calculation have a power of 70% to 90% for the standard deviation ranging from 0.3 to 0.5. Our GDS standard deviation was 0.53 and 0.39 in the placebo and lithium arms, respectively. To the contrary, the between-group difference of GDS may be smaller than the assumed 0.25 and an even larger sample size than originally calculated may have been required to detect a significant difference. Second, 6 month trial duration could not exclude a beneficial effect of lithium on long-term functional worsening. Third, we cannot exclude selection bias as the majority of our participants were unemployed females with significant neurocognitive impairment. Fourth, all our participants were black Xhosa speaking Africans which limits the generalizability of our results.

In summary, we found no additional benefit of adjunctive lithium to placebo in African patients with HAND after 6 months of treatment. Future adjunctive lithium studies should follow-up patients for a longer duration to determine whether lithium has a beneficial effect on HAND progression.

Acknowledgments

The acknowledge the study participants: The rest of the study team: Kareema Poggenpoel (administration support), Laura Comrie (clinical support), Nicky Kramer (study pharmacist), Nozipho Mawisa (study nurse), Pam Jordan (data capturer), Queen Maswana (recruiter), Rasmita Ori (clinical support), Shireen Surtie (study coordinator), Teboho Linda (neuropsychology technician) and Wynand Smythe (study pharmacist).
Members of the DSMB and the trial steering committee: Giovanni Schifitto, Ned Sacktor, Carl Lombard, Rhoderick Machekano, Haylene Nell and Bernd Rosenkranz.
Norgine Pty (Ltd) who unconditionally donated lithium carbonate and identical placebo.

References

[1] Heaton RK, Clifford DB, Franklin DR, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. Neurology 2010;75:2087–96.
[2] Heaton RK, Franklin DR, Ellis RJ, et al. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. J Neurovirol 2011;17:3–16.
[3] Sacktor N, Skolasky RL, Seaberg E, et al. Prevalence of HIV-associated neurocognitive disorders in the Multicenter AIDS Cohort Study. Neurology 2016;86:334–40.
[4] Fabbiani M, Ciccarelli N, Tana M, et al. Cardiovascular risk factors and carotid intima-media thickness are associated with lower cognitive performance in HIV-infected patients. HIV Med 2013;14:136–44.
[5] Cysique LA, Vaida F, Letendre S, et al. Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. Neurology [Internet] 2009;73:342–8.
[6] Cysique LA, Bain MP, Brew BJ, et al. The burden of HIV-associated neurocognitive impairment in Australia and its estimates for the future. Sex Health 2011;8:541–50.
[7] McGuire JL, Barrett JS, Vezina HE, et al. Adjuvant therapies for HIV-associated neurocognitive disorders. Ann Clin Transl Neurol 2014;1:938–52.
[8] Letendre SL, Woods SP, Ellis RJ, et al. Lithium improves HIV-associated neurocognitive impairment. AIDS 2006;20:1885–8.
[9] Schifitto G, Zhong J, Gill D, et al. Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. J Neurovirol [Internet] 2009;15:176–86.
[10] Hajek T, Weiner MW. Neuroprotective effects of lithium in human brain? Food for thought. Curr Alzheimer Res 2016;13:862–72.
[11] Forlenza OV, Aprahamian I, de Paula VJ, Hajek T, et al. Lithium, a therapy for AD: current evidence from clinical trials of neurodegenerative disorders. Curr Alzheimer Res 2016;13:879–86.
[12] Crowder RJ, Freeman RS. Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. J Neurosci 1998;18:2933–43.
[13] Lazzara CA, Kim YH. Potential application of lithium in Parkinson's and other neurodegenerative diseases. Front Neurosci 2015;9:403.
[14] Peineau S, Bradley C, Taghibiglou C, et al. The role of GSK-3 in synaptic plasticity. Br J Pharmacol 2008;153(suppl):S428–37.
[15] Carey CL, Woods SP, Gonzalez R, et al. Predictive validity of global deficit scores in detecting neuropsychological impairment in HIV infection. J Clin Exp Neuropsychol 2004;26:307–19.
[16] Myer L, Smit J, Roux LL, et al. Common mental disorders among HIV-infected individuals in South Africa: prevalence, predictors, and validation of brief psychiatric rating scales. AIDS Patient Care STDS 2008;22:147–58.
[17] Joska JA, Westgarth-Taylor J, Hoare J, et al. Neuropsychological outcomes in adults commencing highly active anti-retroviral treatment in South Africa: a prospective study. BMC Infect Dis 2012;12:39.
[18] Benedetti F, Carlino E, Pollo A. How placebos change the patient's brain. Neuropsychopharmacology 2011;36:339–54.
[19] Murray D, Stoessl AJ. Mechanisms and therapeutic implications of the placebo effect in neurological and psychiatric conditions. Pharmacol Ther 2013;140:306–18.
[20] Holford N. Clinical pharmacology=disease progression + drug action. Br J Clin Pharmacol 2015;79:18–27.
[21] Tedaldi EM, Minniti NL, Fischer T. HIV-associated neurocognitive disorders: The relationship of Hiv infection with physical and social comorbidities. BioMed Res Int 2015;2015:641913.
[22] Hou L, Heilbronner U, Degenhardt F, et al. Genetic variants associated with response to lithium treatment in bipolar disorder: a genome-wide association study. Lancet 2016;387:1085–93.

Table 1. Per protocol analysis of neuropsychological changes

Neuropsychological domain	**Baseline** ***Lithium***	**Baseline** ***Placebo***	***P* value**	**Week 24** ***Lithium***	**Week 24** ***Placebo***	***P* value**
Attention						
Digit Span	6.06 ± 1.27[a]	6.24 ± 1.23[a]	*0.577*[c]	6.33 ± 1.52[a]	6.68 ± 1.38[a]	*0.357*[c]
Paced Auditory Serial Addition Test	15.0 (12.0 – 19.5)[b]	16.00 (13.0 – 22.0)[b]	*0.908*[d]	17.50 (11.0 – 23.0)[b]	16.0 (13.0 – 22.0)[b]	*0.994*[d]
Learning and memory						
Hopkins Verbal Learning Test (recall)	5.0 (5.0 – 6.0)[b]	6.0 (4.0 – 7.0)[b]	*0.319*[d]	7.0 (7.0 – 8.0)[b]	8.0 (6.0 – 9.0)[b]	*0.590*[d]
Motor speed						
Finger Tapping non-dominant hand	7.58 (6.70 – 8.73)[b]	7.83 (6.84 – 8.64)[b]	*0.868*[d]	6.76 (6.26 – 7.87)[b]	7.23 (6.28 – 8.15)[b]	*0.228*[d]
Grooved Pegboard Test non- dominant hand	96.60 (79.96 – 123.04)[b]	101.43 (84.13 – 118.29)[b]	*0.635*[d]	79.79 (75.09 – 95.56)[b]	89.78 (80.94 – 103.86)[b]	*0.025*[d,e]
Psychomotor speed						
Trail Making Test A	59.54 (46.46 – 88.58)[b]	66.62 (51.54 – 83.7)[b]	*0.386*[d]	47.05 (37.2 – 55.79)[b]	49.67 (37.51 – 59.99)[b]	*0.471*[d]
Colour Trails Test 1	58.84 (50.58 – 82.15)[b]	78.07 (58.71 – 95.44)[b]	*0.041*[d]	64.23 (46.58 – 75.87)[b]	69.33 (57.78 – 81.19)[b]	*0.141*[d]
Digit Symbol-Coding	28.84 ± 10.55[a]	27.79 ± 9.22[a]	*0.668*[c]	29.27 ± 9.72[a]	29.58 ± 11.50[a]	*0.909*[c]
Executive function						
Colour Trails Test 2	154.82 ± 45.95[a]	173.98 ± 41.04[a]	*0.078*[c]	143.33 (114.24 – 167.67)[b]	132.95 (119.02 – 163.12)[b]	*0.868*[d]
Stroop Colour-Word test	24.19 ± 8.25[a]	23.88 ± 8.53[a]	*0.883*[c]	26.80 ± 8.89[a]	27.06 ± 8.77[a]	*0.907*[c]
Wisconsin Card-Sorting Test	41.0 (31.0 – 87.5)[b]	42.0 (33.0 – 57.0)[b]	*0.797*[d]	41.0 (30.0 – 61.0)[b]	37.0 (32.0 – 45.0)[b]	*0.473*[d]
Visual learning and memory						
Rey Complex Figure (copy)	21.70 ± 7.87[a]	21.91 ± 8.0[a]	*0.915*[c]	23.0 ± 6.93[a]	20.52 ± 7.86[a]	*0.196*[c]
Rey Complex Figure (3 minutes)	10.44 ± 4.59[a]	10.56 ± 3.89[a]	*0.908*[c]	12.27 ± 5.79[a]	10.37 ± 4.82[a]	*0.169*[c]
Verbal Fluency						
Animals	13.72 ± 2.96[a]	13.71 ± 3.75[a]	*0.988*[c]	13.90 ± 2.51[a]	14.19 ± 2.68[a]	*0.660*[c]
Fruit and Vegetables	14.06 ± 3.05[a]	12.88 ± 2.88[a]	*0.111*[c]	13.83 ± 2.95[a]	13.48 ± 3.34[a]	*0.667*[c]
Depression score						
CES-D	9.0 (4.0 – 17.0)[b]	8.0 (3.0 – 14.0)[b]	*0.672*[d]	2.5 (0 – 7.0)[b]	3.0 (0 – 5.0)[b]	*0.426*[d]
Summary score						
Global Deficit Score	1.08 (0.83 - 1.44)[b]	1.11 (0.82 - 1.53)[b]	*0.793*[d]	0.61 (0.33 - 0.89	0.68 (0.41 - 1.0)	*0.462*[d]

[a]Mean and standard deviation; [b]Median and interquartile range; [c]t-test (2 samples); [d]Wilcoxon sum rank; [e]Not significant p-value after False Discovery Rate (FDR) correction. CES-D = Center for Epidemiologic Studies Depression scale

Table 2. Changes in MRS metabolites

Metabolite	Brain area	Baseline *Lithium*	Baseline *Placebo*	*P* value	Week 23 *Lithium*	Week 23 *Placebo*	*P* value
Glutamate	Cortical: anterior cingulate cortex	8.40 ± 0.95[a] (n=30)	8.21 ± 0.95[a] (n=32)	0.415[c]	8.33 ± 0.60[a] (n=28)	8.01 ± 0.66[a] (n=27)	0.068[c]
	White matter: left frontal white matter	5.09 ± 0.84[a] (n=30)	5.34 ± 0.97[a] (n=23)	0.323[c]	5.07 ± 0.81[a] (n=18)	5.04 ± 0.65[a] (n=17)	0.911[c]
	Deep brain structure: left thalamus	5.20 ± 0.81[a] (n=29)	5.15 ± 0.86[a] (n=30)	0.811[c]	5.09 ± 0.77[a] (n=26)	4.91 ± 0.65[a] (n=23)	0.395[c]
Myo-inositol concentration (mI)	Cortical: anterior cingulate cortex	5.20 ± 0.62[a] (n=30)	5.19 ± 0.56[a] (n=32)	0.921[c]	5.14 ± 0.76[a] (n=28)	5.24 ± 0.70[a] (n=27)	0.607[c]
	White matter: left frontal white matter	4.64 (4.31 - 5.34)[b] (n=30)	5.42 (4.28 - 7.41)[b] (n=23)	0.037[d,e]	4.80 ± 0.67[a] (n=18)	4.76 ± 0.88[a] (n=17)	0.871[c]
	Deep brain structure: left thalamus	4.12 ± 0.70[a] (n=29)	4.16 ± 0.82[a] (n=30)	0.843[c]	4.14 ± 0.67[a] (n=26)	4.43 ± 0.60[a] (n=23)	0.117[c]
N-acetyl-aspartate (NAA)	Cortical: anterior cingulate cortex	6.14 ± 0 .68[a] (n=30)	6.29 ± 0.64[a] (n=32)	0.366[c]	6.27 ± 0.64[a] (n=28)	6.04 ± 0.71[a] (n=27)	0.211[c]
	White matter: left frontal white matter	5.46 ± 0.86[a] (n=30)	5.51 ± 0.86[a] (n=23)	0.855[c]	5.46 ± 0.61[a] (n=18)	5.30 ± 1.01 (n=17)	0.565[c]
	Deep brain structure: left thalamus	6.64 (5.99 - 6.95)[b] (n=29)	6.64 (5.46 - 7.05)[b] (n=30)	0.886[d]	6.58 (6.28 - 6.92)[b] (n=26)	6.69 (5.98 -7.37)[b] (n=23)	0.609[d]
Choline (Cho)	Cortical: anterior cingulate cortex	1.38 ± 0 .22[a] (n=30)	1.40 ± 0.15[a] (n=32)	0.749[e]	1.42 (1.30 - 1.57)[b] (n=28)	1.33 (1.21 - 1.45)[b] (n=27)	0.114[d]
	White matter: left frontal white matter	1.34 ± 0.24[a] (n=30)	1.37 ± 0.23[a] (n=23)	0.695[d]	1.33 (1.21 - 1.47)[b] (n=30)	1.39 (1.17 - 1.52)[b] (n=23)	0.632[d]
	Deep brain structure: left thalamus	1.38 ± 0.24[a] (n=29)	1.37 ± 0.17[a] (n=30)	0.777[c]	1.45 ± 0.18[a] (n=26)	1.36 ± 0.13[a] (n=23)	0.071[c]
N-acetyl-asparate with N-acetyl-aspartyl-glutamate (NAA+NAAG)	Cortical: anterior cingulate cortex	6.51 ± 0.73[a] (n=30)	6.47 ± 0.60[a] (n=32)	0.818[c]	6.64 ± 0.55[a] (n=28)	6.36 ± 0.52[a] (n=27)	0.058[c]
	White matter: left frontal white matter	6.08 ± 0.58[a] (n=30)	6.00 ± 0.51[a] (n=23)	0.602[c]	5.81 ± 0.50[a] (n=18)	5.88 ± 0.67[a] (n=17)	0.728[c]
	Deep brain structure: left thalamus	6.91 (6.82 - 7.15)[b] (n=29)	7.08 (6.41 - 7.42) (n=30)	0.549[d]	6.94 ± 0.50[a] (n=26)	7.07 ± 0.73[a] (n=23)	0.819[c]
Creatine (Cr)	Cortical: anterior cingulate cortex	1.38 ± 0.22[a] (n=30)	1.40 ± 0.15[a] (n=32)	0.749[e]	1.49 ± 0.34[a] (n=28)	1.35 ± 0.21[a] (n=27)	0.086[e]
	White matter: left frontal white matter	4.53 ± 0.52[a] (n=30)	4.54 ± 0.66[a] (n=23)	0.932[c]	4.36 ± 0.37[a] (n=18)	4.43 ± 0.56[a] (n=17)	0.634[c]
	Deep brain structure: left thalamus	4.97 ± 0.52[a] (n=29)	4.92 ± 0.57[a] (n=30)	0.736[c]	5.02 (4.72 - 5.31)[b] (n=26)	4.78 (4.55 - 5.25)[b] (n=23)	0.357[d]
Glutamate with glutamine (Glx)	Cortical: anterior cingulate cortex	10.35 ± 1.95[a] (n=30)	10.31 ± 1.76[a] (n=32)	0.928[c]	10.91 (9.92 - 11.96)[b] (n=28)	9.50 (8.31 - 11.51)[b] (n=27)	0.916[d]
	White matter: left frontal white matter	6.52 ± 1.63[a] (n=30)	6.75 ± 1.29[a] (n=23)	0.589[c]	6.30 ± 1.42[a] (n=18)	6.64 ± 1.21[a] (n=17)	0.451[c]
	Deep brain structure: left thalamus	6.68 ± 1.68[a] (n=29)	6.31 ± 1.60[a] (n=30)	0.382[c]	6.23 ± 1.00[a] (n=26)	6.17 ± 1.53[a] (n=23)	0.884[e]

[a]Mean and standard deviation; [b]Median and interquartile range; [c]t-test (2 samples); [d]Wilcoxon sum rank; [e]t-test (2 samples for unequal variances); [e]Not significant p-value after False Discovery Rate (FDR) correction; mI = Myo-inositol concentration; NAA = N-acetyl-aspartate; Cho = Choline; NAA+NAAG = N-acetyl-asparate with N-acetyl-aspartyl-glutamate; Cr = Creatine; Glx = Glutamate with glutamine

Table 3. Serious adverse events

Serious adverse event	Relationship to study drug	Lithium (n=32)	Placebo (n=34)
Orbital cellulitis secondary to bacterial sinusitis. *Description: The complicated bacterial sinusitis was diagnosed after enrolment, but prior to study medication dosing. The infection resolved with appropriate intravenous antibiotic therapy.*	Not related	1 (3.1%)	0
Severe major depressive disorder (MDD) episode without psychotic features, complicated by an alcohol use disorder. *Description: Both participants were not considered to have an alcohol use disorder upon screening, using the Alcohol Use Disorders Identification Test.*(23) *However, the clinical presentation of the MDD episode during the trial was considered severe enough in both participants to warrant withdrawal from the study and referral for further psychiatric care.*	Not related	0	2 (5.9%)
Pelvic inflammatory disease (PID) requiring hospital admission. *Description: The participant presented to a medical facility with lower abdominal pain and PID symptoms severe enough to require intravenous antibiotic therapy. The infection resolved after appropriate inpatient treatment.*	Not related	1 (3.1%)	0
Post-lumbar puncture headache requiring hospital admission. *Description: Consenting participants underwent cerebrospinal fluid collection for research purposes (not reported in this paper). The participant presented 5 days later with symptoms suggestive of meningitis. Meningitis was excluded and the participant was successfully treated with analgesics.*	Not related	1 (3.1%)	0
Medically significant but asymptomatic hypochromic microcytic anaemia. *Description: The participant presented at the last visit with an asymptomatic hypochromic microcytic anaemia of 5.9 g/dL. Vitamin B12 deficiency, hypothyroidism and alcohol abuse were excluded. Further workup was not possible as the participant relocated and care was handed over to her local practitioner. At enrolment, the participant was noted to have been previously treated for a mild iron deficiency anaemia secondary to menorrhagia.*	Not related	1 (3.1%)	0

CHAPTER 6

Renal safety of lithium in HIV-infected patients established on tenofovir disoproxil fumarate containing antiretroviral therapy: analysis from a randomised placebo-controlled trial.

Decloedt *et al. AIDS Res Ther (2017) 14:6*
DOI 10.1186/s12981-017-0134-2

AIDS Research and Therapy

RESEARCH Open Access

Renal safety of lithium in HIV-infected patients established on tenofovir disoproxil fumarate containing antiretroviral therapy: analysis from a randomized placebo-controlled trial

Eric H. Decloedt[1,4*], Maia Lesosky[3,5], Gary Maartens[4] and John A. Joska[2]

Abstract

Background: The prevalence of bipolar disorder in HIV-infected patients is higher than the general population. Lithium is the most effective mood stabiliser, while tenofovir disoproxil fumarate (TDF) is frequently used as part of combination antiretroviral therapy (ART). Both TDF and lithium are associated with renal tubular toxicity, which could be additive, or a pharmacokinetic interaction may occur at renal transporters with a decrease in TDF elimination.

Objective: We report on the change in estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease formula in participants who received ART including TDF and were enrolled in a 24 week randomised trial of lithium versus placebo in patients with HIV-associated neurocognitive impairment.

Methods: We included HIV-infected adults with cognitive impairment established on ART for at least 6 months with a suppressed viral load attending public sector ART clinics in Cape Town, South Africa. We excluded participants with an eGFR <60 mL/min and treated with medications predisposing to lithium toxicity. We reviewed participants weekly for the first month for adverse events followed by 4 weekly visits for renal function assessment, adverse event monitoring and adherence. Lithium dose was titrated to achieve the maintenance target plasma concentration of between 0.6 and 1.0 mmol/L. Sham lithium concentrations were generated for participants receiving placebo.

Results: We included 23 participants allocated to the lithium arm and 30 participants allocated to the placebo arm. Baseline characteristics were not statistically different with a mean age of 37.7 and 40.8 years, a median time on ART of 33 and 40 months and an eGFR of 139.3 and 131.0 mL/min in the lithium and placebo arms respectively. There was no statistical significant difference in the reduction in eGFR or increase in potassium between the two arms during the 24 weeks.

Conclusions: We found that 24-week treatment of HIV-infected patients with lithium and TDF did not result in increased nephrotoxicity.

Trial registration The study was registered on the Pan African Clinical Trials Registry (PACTR) with the identifier number PACTR201310000635418. Registered 11 October 2013 before the first participant was enrolled

Keywords: South Africa, Randomised placebo-controlled clinical trial, HIV-associated neurocognitive impairment, HIV, Lithium, Placebo, Antiretroviral therapy, Tenofovir

*Correspondence: ericdecloedt@sun.ac.za
[1] Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa
Full list of author information is available at the end of the article

Background

The prevalence of bipolar disorder in human immunodeficiency virus (HIV)-infected patients is 4–5 times higher than the general population [1, 2]. The most effective mood stabiliser is lithium while the nucleotide reverse transcriptase inhibitor tenofovir disoproxil fumarate (TDF) is frequently used as part of combination antiretroviral therapy (ART) [3]. Both TDF and lithium are associated with renal tubular toxicity, which could be additive [4, 5]. Furthermore, a TDF-lithium pharmacokinetic interaction may occur at renal transporters. TDF is eliminated via proximal tubular secretion and renal toxicity is thought to be related to accumulation of intracellular tenofovir in the proximal tubular cell [6]. Intracellular TDF inhibits mitochondrial deoxyribonucleic acid (DNA) polymerase gamma with DNA depletion and oxidative respiratory chain dysfunction [7]. Mitochondrial dysfunction impairs tubular reabsorption of ions and molecules causing a Fanconi-like syndrome, or may lead to cell apoptosis and acute tubular necrosis [7]. TDF is a substrate of a number of transporters at the proximal renal tubule. The organic anion transporter-1 (OATP-1) transports TDF intracellularly while the multidrug resistance protein 4 (MRP-4) mediates active secretion from the tubular cell [8]. In rats lithium impairs OATP-1 function, which may protect against TDF renal toxicity [9]. Twenty to eighty seven percent of lithium treated patients develop a reduction in urinary concentrating ability within weeks after starting treatment [10]. Lithium-induced nephrogenic diabetes insipidus (NDI) is thought to be caused by downregulation of intracellular calcium signalling with inhibition of glycogen synthase kinase-3-beta (GSK-3-ß), resulting in a number of downstream effects including decreased aquaporin-2 expression [11]. Recently it was shown that MRP-1 expression is regulated by GSK-3-β, suggesting that lithium may decrease MRP expression and predispose to TDF renal toxicity [12]. The proximal tubule as a common site for TDF and lithium renal toxicity may further contribute to renal toxicity. Lithium-induced renal toxicity may involve any segment of the nephron or kidney although the distal tubule seems to be involved in NDI [13]. There is currently no published data on the renal safety of concomitant TDF and lithium.

We previously published the results of a 24 week randomised placebo-controlled trial to study lithium as an adjunctive pharmacotherapy in patients with moderate to severe HIV-associated neurocognitive impairment [14]. In this study we report changes in the estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease (MDRD) formula as well as changes in potassium in participants who received ART including TDF who were randomised to lithium or placebo.

Methods

Our methodology has been previously published, but in brief we included HIV-infected adults (≥18 and ≤70 years), established on ART for at least 6 months with a suppressed viral load (HIV RNA <400 copies/mL) with cognitive impairment attending public sector ART clinics in Cape Town, South Africa [14]. We dosed lithium carbonate 250 mg tablets (Camcolit®, manufactured by Norgine) and matching placebo (manufactured by Norgine). We excluded participants who used medications that may predispose to lithium toxicity (diuretics, angiotensin converting enzyme inhibitors or angiotensin receptor blockers and non-steroidal anti-inflammatory medicines), participants with an eGFR of less than 60 mL/min and dehydrated participants with diarrhoea. We reviewed participants weekly for first month followed by 4 weekly visits for adverse events and adherence. After screening and study drug initiation, we repeated renal function (sodium, potassium, urea and creatinine) at weeks 4, 8, 12, 16, 20 and 24. Some participants switched treatment to TDF during the study period, and for this analysis we only included patients who received TDF for the full 24 weeks. Lithium dose was titrated assuming linear pharmacokinetics to achieve the maintenance target plasma concentration of lithium in patients with bipolar mood disorder of between 0.6 and 1.0 mmol/L. Sham lithium concentrations were generated for participants receiving placebo by the study statistician who was unblinded to treatment allocation. After each visit an investigator not directly responsible for participant follow-ups received a log with the participant number, blinded lithium concentration from the study statistician (real or sham), current study drug dose and any adverse events noted by other investigators. Based on the information the investigator recommended lithium and sham dose adjustments for implementation.

Results

We included 53 participants in this analysis with 23 participants allocated to the lithium arm and 30 participants allocated to the placebo arm. Baseline characteristics between the 2 arms were similar and are described in Table 1. Adherence in both treatment arms were similar and reported previously [14]. The proportion of patients with lithium concentrations in the therapeutic range are presented in Fig. 1. Three participants allocated to the lithium arm developed symptoms of NDI (polyuria) which resolved on dose reduction ($p = 0.042$ compared

Table 1 Baseline characteristics

Baseline characteristic	Lithium (n = 23)	Placebo (n = 30)	p value
Gender			
Male	n = 1 (4%)	n = 4 (13%)	0.374[e]
Female	n = 22 (96%)	n = 26 (87%)	
Age	37.7 ± 8.1[a] years	40.8 ± 8.54[a] years	0.186[c]
Weight	68.5 ± 16.2[a] kg	71.2 ± 12.7[a] kg	0.493[c]
Months on ART	33 (12–56)[b] months	40 (26–68)[b] months	0.262[d]
Renal function			
Creatinine	58 (49–62)[b] µmol/L	58.5 (50–68)[b] µmol/L	0.404[d]
eGFR MDRD	139.3 (118.1–159.7)[b] mL/min	131.0 (110.9–156.9)[b] mL/min	0.572[d]

eGFR estimated glomerular filtration rate, *MDRD* modification of diet in renal disease formula

[a] Mean and standard deviation

[b] Median and interquartile range

[c] t-test (2 samples)

[d] Wilcoxon sum rank

[e] Fisher's exact test

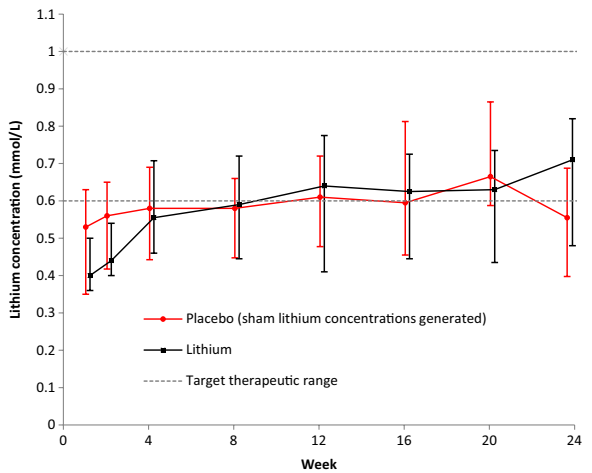


Fig. 1 The graph shows the mean change in lithium concentrations. The *bars* indicate median and interquartile range change in lithium concentrations over the 24 weeks in the lithium and placebo arms respectively. Sham lithium concentrations were generated for the placebo arm. The *broken line* indicates the target therapeutic range of 0.6–1.0 mmol/L

to placebo arm). No participant allocated to the placebo arm developed symptoms of NDI. Change in eGFR, creatinine and potassium were similar between the 2 arms (see Figs. 2, 3, 4). There was no statistical significant difference between the two arms in the proportion of participants who had a reduction in eGFR (see Table 2). There was no statistically significant difference in the eGFR slope between the 2 treatment arms (see Fig. 2a) (p value = 0.06) when using linear regression.

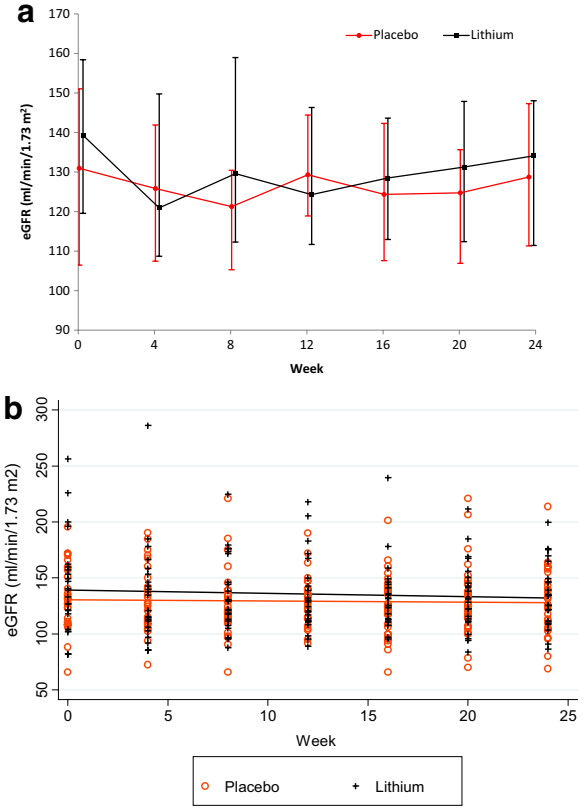


Fig. 2 **a** The graph shows the mean estimated glomerular filtration rate (eGFR) as calculated using the modification of diet in renal disease (MDRD). The *bars* indicate median and interquartile range change in eGFR over the 24 weeks in the lithium and placebo arms respectively. **b** Scatter plot of estimated glomerular filtration rate (eGFR) as calculated using the modification of diet in renal disease (MDRD) over the 24 weeks in the lithium and placebo arms respectively. The *solid lines* indicate the linear regression lines for the lithium and placebo arms respectively. Treatment allocation did not have a statistically significant effect on eGFR (p value 0.06)

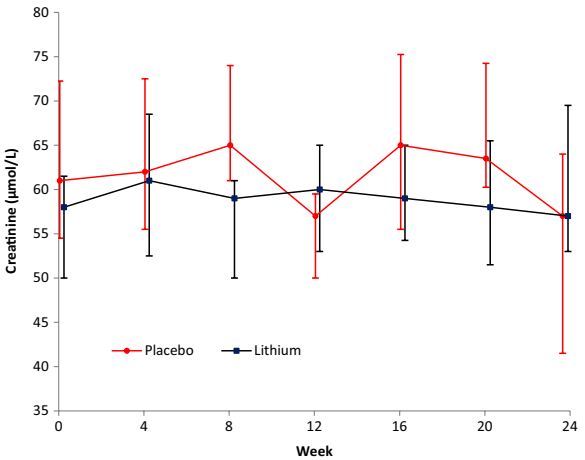


Fig. 3 The graph shows the mean change in creatinine (µmol/L). The *bars* indicate median and interquartile range of creatinine over the 24 weeks in the lithium and placebo arms respectively

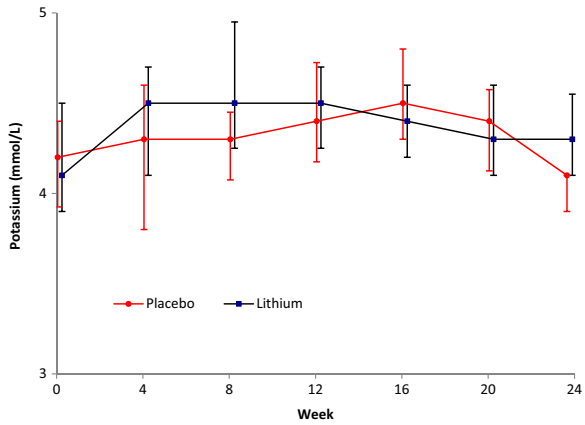


Fig. 4 The graph shows the mean change in potassium. The *bars* indicate median and interquartile range change in potassium over the 24 weeks in the lithium and placebo arms respectively

Discussion

We reported the renal safety of lithium co-administered with TDF as part of a 24 week randomised placebo-controlled trial. To the best of our knowledge we described the first safety data of co-administered lithium with TDF. We found that lithium and TDF co-administration did not increase the risk of renal impairment in HIV-infected patients with neurocognitive impairment and preserved renal function over a 24-week period.

NDI is a well-recognised early side effect of lithium administration. Lithium causes dysregulation of the aquaporin-2 water channels in the collecting ducts with impaired pro-urine concentration ability [13, 15]. Three patients in the lithium arm developed NDI which resolved with a lithium dose reduction. Lithium-induced nephrotoxicity has been long recognised, but the extent and risk factors required to frame a risk-benefit profile for patients has been much debated [16]. A recent population-based study in psychiatric patients with lithium exposure found that monthly eGFR decline was similar in the lithium and reference group after adjusting for co-morbidities, concomitant medication and episodes of lithium toxicity [17]. Our findings in a young cohort with no lithium toxicity episodes and limited treatment duration echo these findings.

Our study has several limitations. First, we reported on the safety of lithium dosed with TDF in a randomised placebo-controlled trial that was not powered for this endpoint. Second, we followed patients for 24 weeks and we can only make inferences about the short-term safety of concomitant lithium and TDF administration. Third,

Table 2 Estimated glomerular filtration rate change

eGFR MDRD change[d]	Treatment arm	Week 4 % (n)	Week 8 % (n)	Week 12 % (n)	Week 16 % (n)	Week 20 % (n)	Week 24 % (n)
Increased	Lithium	30.4% (7/23)	39.1% (9/23)	39.1% (9/23)	34.8% (8/23)	39.1% (9/23)	56.5% (13/23)
	Placebo	40% (12/30)	50% (15/30)	43.3% (13/30)	33.3% (10/30)	43.3% (13/30)	46.7% (14/30)
		p = 0.518[a]	p = 0.621[a]	p = 0.095[a]	p = 0.012[a]	p = 0.095[a]	p = 0.506[a]
Grade 1 Decreased 0.1% to <10% from baseline	Lithium	34.8% (8/23)	13% (3/23)	8.7% (2/23)	26.1% (6/23)	17.4% (4/23)	8.7% (2/23)
	Placebo	23.3% (7/30)	16.7% (5/30)	20% (6/30)	23.3% (7/30)	30% (9/30)	20% (6/30)
		p = 0.495[a]	p = 0.264[b]	p = 0.229[b]	p = 0.817[a]	p = 0.233[b]	p = 0.229[b]
Grade 2 Decreased 10 to <30% from baseline	Lithium	26.0% (6/23)	26.1% (9/23)	43.5% (10/23)	21.7% (5/23)	34.8% (8/23)	23% (6/23)
	Placebo	26.7% (8/30)	13.3% (5/30)	30% (9/30)	33.3% (10/30)	13.3% (4/30)	30% (9/30)
		p = 0.971[a]	p = 0.164[a]	p = 0.765[a]	p = 0.484[a]	p = 0.145[a]	p = 0.814[b]
Grade 3 Decreased ≥30 to <50% from baseline	Lithium	8.7% (2/23)	8.7% (2/23)	8.7% (2/23)	8.7% (2/23)	8.7% (2/23)	8.7% (2/23)
	Placebo	0%	0%	3.3% (1/30)	0%	13.3% (4/30)	3.3% (1/30)
		p = 0.202[b]	p = 0.202[b]	p = 0.418[b]	p = 0.202[b]	p = 0.493[b]	p = 0.418[b]
Grade 4 Decreased ≥50% from baseline	Lithium	0%	0%	0%	0%	0%	0%
	Placebo	0%	0%	0%	3.3% (1/30)	0%	0%
					p = 574[b]		
Creatinine not measured[c]	Lithium	0%	0%	0%	4.3% (1/23)	0%	0%
	Placebo	0%	10% (3/30)	3.3% (1/30)	3.3% (1/30)	0%	0%
			p = 0.197[b]	p = 0.574[b]	p = 0.687[b]		

eGFR estimated glomerular filtration rate, *MDRD* modification of diet in renal disease formula

[a] Chi-squared test

[b] One-sided Fisher's exact test

[c] Participants did not attend the specific study visit

[d] Grading according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (Version 2.0 November 2014)

we may have missed more subtle markers of tubulopathy as we did not measure urine markers of tubulopathy. Fourth, only approximately half of participants had therapeutic lithium trough concentrations. We collected lithium trough concentrations as soon as participants arrived at the study site and despite best efforts, sample collection time for some participants was beyond 12 h. Last, we excluded patients with renal impairment and concomitant medication which may potentiate lithium toxicity.

We could not rule out nephrotoxicity of long-term concomitant treatment of TDF and lithium and future research should focus on the long-term follow-up of TDF-treated HIV-infected patients with lithium-treated bipolar disorder.

Conclusions

We found that 24-week treatment of HIV-infected patients with lithium and TDF, preserved renal function and no episodes of lithium toxicity did not result in increased nephrotoxicity. To the best of our knowledge we described the first safety data of co-administered lithium with TDF. Our finding supports the renal safety of TDF-based ART in HIV-infected patients with bipolar disorder requiring lithium therapy as a mood stabiliser.

Abbreviations

ART: antiretroviral therapy; DNA: deoxyribonucleic acid; eGFR: estimated glomerular filtration rate; GSK-3-β: glycogen synthase kinase-3-beta; HIV: human immunodeficiency virus; MDRD: modification of diet in renal disease; MRP-4: multi-drug resistance protein 4; NDI: nephrogenic diabetes insipidus; OATP-1: organic anion transporter-1; TDF: tenofovir disoproxil fumarate.

Authors' contributions

ED: study concept and design, analysis and interpretation of data, drafting and revising the manuscript for content, acquisition of data, study supervision and obtaining funding. ML: analysis and interpretation of data, statistical analysis. GM: study concept and design, revising the manuscript for content and obtaining funding. JJ: study concept and design, revising the manuscript for content, study supervision and obtaining funding. ML: statistical analysis. All authors read and approved the final manuscript.

Author details

[1] Division of Clinical Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa. [2] Division of Neuropsychiatry, Department of Psychiatry and Mental Health, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa. [3] Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa. [4] Division of Clinical Pharmacology, Department of Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa. [5] Department of Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.

Acknowledgements

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was registered on the Pan African Clinical Trials Registry (PACTR) with the identifier number PACTR201310000635418. Registered 11 October 2013 before the first participant was enrolled.

The study was approved by the human research ethics committees of the University of Cape Town (071/2013) and Stellenbosch University (M13/07/027). All participant signed informed consent prior to participation in the study.

Funding

Norgine Pty (Ltd) unconditionally donated lithium carbonate and identical placebo. Norgine Pty (Ltd) had no input in any aspect of the study.

This study was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP Grant Number SP.2011.41304.065).

Received: 8 November 2016 Accepted: 13 January 2017
Published online: 04 February 2017

References

1. Moore DJ, Posada C, Parikh M, et al. HIV-infected individuals with co-occurring bipolar disorder evidence poor antiretroviral and psychiatric medication adherence. AIDS Behav. 2012;16(8):2257–66. doi:10.1007/s10461-011-0072-2.
2. de Sousa Gurgel W, da Silva Carneiro AH, Barreto Rebouças D, et al. Prevalence of bipolar disorder in a HIV-infected outpatient population. AIDS Care. 2013;25(12):1499–503. doi:10.1080/09540121.2013.779625.
3. Miura T, Noma H, Furukawa TA, et al. Comparative efficacy and tolerability of pharmacological treatments in the maintenance treatment of bipolar disorder: a systematic review and network meta-analysis. Lancet Psychiatry. 2014;1(5):351–9. doi:10.1016/S2215-0366(14)70314-1.
4. Shine B, McKnight RF, Leaver L, Geddes JR. Long-term effects of lithium on renal, thyroid, and parathyroid function: a retrospective analysis of laboratory data. Lancet (London, England). 2015;386(9992):461–8. doi:10.1016/S0140-6736(14)61842-0.
5. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis. 2010;51(5):496–505. doi:10.1086/655681.
6. Kohler JJ, Hosseini SH, Hoying-Brandt A, et al. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. Lab Invest. 2009;89(5):513–9. doi:10.1038/labinvest.2009.14.
7. Tourret J, Deray G, Isnard-Bagnis C. Tenofovir effect on the kidneys of HIV-infected patients: a double-edged sword? J Am Soc Nephrol. 2013;24(10):1519–27. doi:10.1681/ASN.2012080857.
8. Kohler JJ, Hosseini SH, Green E, et al. Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. Lab Invest. 2011;91(6):852–8. doi:10.1038/labinvest.2011.48.
9. Uwai Y, Tsuge M, Tokai Y, Kawasaki T, Nabekura T. Lithium interferes with the urinary excretion of phenolsulfonphthalein in rats: involvement of a reduced content of α-ketoglutarate, the driving force for organic anion transporters OAT1 and OAT3, in the kidney cortex. Pharmacology. 2015;96(5–6):278–83. doi:10.1159/000441253.
10. Azab AN, Shnaider A, Osher Y, Wang D, Bersudsky Y, Belmaker RH. Lithium nephrotoxicity. Int J Bipolar Disord. 2015;3(1):28. doi:10.1186/s40345-015-0028-y.
11. Rej S, Pira S, Marshe V, et al. Molecular mechanisms in lithium-associated renal disease: a systematic review. Int Urol Nephrol. 2016. doi:10.1007/s11255-016-1352-6.
12. Kim H-R, Lee K-Y, Lee K-Y, et al. Transcriptional regulation, stabilization, and subcellular redistribution of multidrug resistance-associated protein 1 (MRP1) by glycogen synthase kinase 3αβ: novel insights on modes of cadmium-induced cell death stimulated by MRP1. Arch Toxicol. 2015;89(8):1271–84. doi:10.1007/s00204-014-1381-9.

13. Alsady M, Baumgarten R, Deen PMT, de Groot T. Lithium in the kidney: friend and foe? J Am Soc Nephrol. 2016;27(6):1587–95. doi:10.1681/ASN.2015080907.
14. Decloedt EH, Freeman C, Howells F, Casson-Crook M, Lesosky M, Koutsilieri E, Lovestone S, Maartens G, Joska J. Moderate to severe HIV-associated neurocognitive impairment: a randomized placebo-controlled trial of lithium. Medicine (Baltimore). 2016;95(45):e5401.
15. McKnight RF, Adida M, Budge K, Stockton S, Goodwin GM, Geddes JR. Lithium toxicity profile: a systematic review and meta-analysis. Lancet (London, England). 2012;379(9817):721–8. doi:10.1016/S0140-6736(11)61516-X.
16. Davis JM, Rosenbaum A, Shahinian V, Brosius FC. Prevention of lithium-associated renal failure: recent evidence. Lancet Psychiatry. 2015;2(12):1045–7. doi:10.1016/S2215-0366(15)00498-8.
17. Clos S, Rauchhaus P, Severn A, Cochrane L, Donnan PT. Long-term effect of lithium maintenance therapy on estimated glomerular filtration rate in patients with affective disorders: a population-based cohort study. Lancet Psychiatry. 2015;2(12):1075–83. doi:10.1016/S2215-0366(15)00316-8.

CHAPTER 7

Summary and conclusions

HIV-associated neurocognitive disorders (HAND) persist, with the prevalence increasing, despite antiretroviral therapy (ART).[1,2] Local data from Cape Town found that while patients with severe cognitive impairment prior to ART initiation show the most cognitive improvement, 23% - 45% of patients remain cognitively impaired after 1 year on ART.[3] The social and economic burden of HAND is enormous. HAND is associated with a range of impairments of daily activities, including employment, driving and medication adherence related to a combination of neurocognitive and neurological impairment.[1] HAND will continue to place an increasing burden on health resources, especially as those living with HIV age and require residential care.[4] This thesis investigated the treatment of patients with moderate to severe HAND. First, we investigated pharmacogenetic and pharmacokinetic data on CNS penetration of efavirenz-tenofovir-emtricitabine. Second, we investigated pharmacokinetic-pharmacodynamic relationships between CSF efavirenz (EFV), 8-hydroxy-efavirenz (8-OH-EFV), tenofovir (TFV) and emtricitabine (FTC) exposure and neurocognitive performance. Third, we evaluated the efficacy of lithium as adjunctive therapy for HAND in a randomised controlled trial (RCT). Final, we investigated whether lithium causes additive nephrotoxicity in combination with TFV.

In **Chapter 4** we reported on the genetic polymorphisms associated with CSF transfer of EFV, 8-OH-EFV, TFV and FTC in Black South Africans. To our knowledge this is the largest published study examining pharmacogenetic, pharmacokinetic and pharmacodynamic associations with CSF EFV-TFV-FTC. This study included 47 adult participants with and without HAND on ART for at least 6 months. We identified novel genetic associations. First, the individual polymorphisms *CYP2B6* 516G→T and 983T→C as well as the composite *CYP2B6* 15582/516/983 genotype in univariate analyses were most strongly associated with the log_{10}-transformed concentrations of plasma EFV, plasma 7-OH-EFV, plasma 8-OH-EFV/EFV ratio and CSF EFV. To the best of our knowledge, we are the first to describe the association

of the composite *CYP2B6* 15582/516/983 genotype with plasma 7-OH-EFV, plasma 8-OH-EFV/EFV ratio and CSF EFV exposure.[5–7] Second, we found that *CYP2A6* polymorphisms (-48A→C and rs10853742) are associated with lower plasma 7-OH-EFV concentrations after adjusting for *CYP2B6* 516G→T, 983T→C, 15582C→T while others have found no association with EFV metabolites.[6,7] The CYP2A6 metabolism pathway of 7-OH-EFV becomes more relevant in patients with impaired CYP2B6 metabolism.[8] We found that the *CYP2B6* rs2279345 polymorphism was independently associated with lower plasma 7-OH-EFV/EFV ratio, which suggests that in addition to the loss of function polymorphisms in *CYP2B6* 516G→T, 983T→C, 15582C→T, this polymorphism also impairs 7-OH-EFV metabolism via CYP2B6. Third, we found that *ABCB1* rs115780656 was independently associated with lower plasma 7-OH-EFV concentrations. Polymorphisms in *ABCB1* have not been conclusively associated with EFV concentrations and its association with 7-OH-EFV and 8-OH-EFV have not been assessed previously.[9–11] Finally, we found that the *CYP2A6* -48A→C polymorphism was independently associated with higher CSF 8-OH-EFV/EFV ratio. It may be possible that the *CYP2A6* -48A→C polymorphism may predispose *CYP2B6* slow metabolizers to higher CSF 8-OH-EFV concentrations and worsen neurocognitive performance.

In **Chapter 5** we investigated lithium as adjunctive therapy in patients with moderate to severe HAND and found that lithium was well tolerated but had no observable benefit on neurocognitive impairment compared with placebo. The 2 pilot studies by Letendre *et al* and Schifitto *et al* who enrolled n=8 and n=15 participants respectively, had no comparator arm, used a fixed lithium dose of 300mg 12 hourly or titrated the dose to 0.4 – 0.8 mmol/l and a had treatment duration of 10 – 12 weeks.[12,13] Our study had a number of advances compared to the 2 pilot studies. Our study duration was 24 weeks, the design was an RCT and the study drugs were dose adjusted using therapeutic drug monitoring with a target range used in the treatment of bipolar

mood disorder (0.6 to 1.0 mmol/l). We considered a number of explanations for our findings. First, the placebo effect is a well described response accompanied by psychobiological changes in the brain.[14] Clinicians are held in high regard and could have biased our participants' expectations and response.[15] Second, participants may have become more familiar with the neuropsychological assessments leading to a practice effect. We deliberately scheduled the neuropsychological assessments 6 months apart to limit a potential practice effect, but cannot completely exclude some practice effect. In addition, no participant underwent a neuropsychological assessment prior to enrolment into this study. Third, we assessed endpoints only twice 6 months apart which prevents a longitudinal description of natural disease progression, placebo response and lithium effect. The trajectory of natural disease, placebo and lithium would be best described in longer term studies where quantitative modelling is applied.[16] It is possible that the placebo response may be temporary. Fourth, cognitive assessment is influenced by HIV infection, physical -, psychiatric -, and social comorbidity.[17] We monitored HIV -, physical- and psychiatric comorbidities, but it is plausible that we missed social comorbidity improvement explained by trial participation. Fifth, it is possible that only patients with certain characteristics (such as depression co-morbidity) may respond significantly better to lithium compared with placebo. Recently a genome-wide association between lithium response and common genetic variants on chromosome 21 has been identified in patients with bipolar disorder.[18] Finally, Schifitto *et al* found no neurocognitive improvement after 10 weeks in 13 participants but a decrease in glutamate with glutamine (Glx) metabolites (an intracellular neurotransmitter marker) in the frontal grey matter and on neuroimaging.[13] It is possible that lithium may have a limited effect on neuronal plasticity and rather decrease brain activation similar to patients with bipolar mania.[19] Although there are compelling mechanistic hypothesis and clinical evidence suggesting the lithium may be protective or treat Alzheimer's Disease (AD), this may not apply to HAND.[20,21] There are similarities in the CSF

biomarker profile of AD and patients with HIV-associated dementia, but also distinct differences eluding to different pathogenic pathways in AD and HIV neuronal injury.[22]

Lithium-induced nephrotoxicity has been long recognised, but the extent and risk factors required to frame a risk-benefit profile for patients have been much debated.[23] In **Chapter 6** we assessed whether lithium causes additive nephrotoxicity in combination with TFV. Both TFV and lithium are associated with renal tubular toxicity, which could be additive.[24,25] The proximal tubule is a common site for TFV and lithium renal toxicity. In addition, a TFV-lithium pharmacokinetic interaction may occur at renal transporters. TFV is eliminated via proximal tubular secretion and renal toxicity is thought to be related to TFV proximal tubular cell accumulation which inhibits mitochondrial deoxyribonucleic acid (DNA) polymerase gamma with DNA depletion and oxidative respiratory chain dysfunction.[26,27] The organic anion transporter-1 (OATP-1) transports TFV intracellularly while the multi-drug resistance protein 4 (MRP-4) mediates active secretion from the tubular cell.[28] MRP-1 expression is regulated by glycogen synthase kinase-3-beta, suggesting that lithium may decrease MRP expression and predispose to TFV renal toxicity.[29] To the best of our knowledge we described the first safety data of co-administered lithium with TFV. We reviewed the renal function of participants weekly for the first month followed by monthly monitoring. We included 53 participants (23 lithium arm and 30 placebo arm) enrolled in the RCT who received TFV as part of their ART. We found no statistical significant difference in the reduction in estimated glomerular filtration rate (eGFR) or increase in potassium between the two arms during the 24 weeks. A population-based study in psychiatric patients with lithium exposure found that monthly eGFR decline was similar in the lithium and reference group after adjusting for co-morbidities, concomitant medication and episodes of lithium toxicity.[30] Our findings in a young cohort with no lithium toxicity episodes and limited treatment duration echo these findings.

Limitations of the thesis studies

The study in **Chapter 4** only consisted of 47 participants and had limited power to detect genetic associations between infrequent genotypes with small effect sizes (increase in plasma or CSF concentrations). For example, the *CYP2A6* -48A→C polymorphism has been associated with increased plasma EFV concentrations in *CYP2B6* slow metabolizers, but we found no association as there only 3 participants with *CYP2B6* slow metabolizer genotype that carried a single *CYP2A6* -48A→C allele.[31] This may have also limited our ability to detect associations between *CYP2B6* 15582C→T and plasma EFV concentrations, as 15582CT heterozygosity has been associated with small increases in plasma EFV exposure, and there were no 15582TT homozygotes in our study.[32] We were not able to detect pharmacokinetic-pharmacodynamic associations, but this may also be due to limited power to detect smaller differences in cognitive impairment. Second, our study was cross-sectional. Neurocognitive changes would have been better assessed longitudinally. Third, we did not measure the unbound concentrations of EFV-TFV-FTC. EFV is 99.8% protein bound, but protein-free EFV concentrations are equivalent in blood plasma and CSF.[33] TFV and FTC is less than 7% and 4% protein bound, respectively.[34] We assessed CSF-to-plasma ratios of total concentrations. Protein-free CSF-to-plasma concentrations of EFV in particular may have more accurately reflected the pharmacodynamically active concentrations. Fourth, we did not measure the phase II EFV metabolites in CSF, which exceed concentrations of CSF EFV and CSF 8-OH-EFV.[7] The effect of the phase II EFV metabolites on neurocognition are unknown. Last, our study only included adults. Our pharmacokinetic and pharmacodynamic findings can therefore not be applied to a paediatric population. There are concerns about the limited safety data of EFV on the neurocognitive development of children and our lack of an neurocognitive association with EFV-TFV-FTC pharmacokinetics in adults cannot be applied to children.[35]

We were unable to enroll our original calculated sample in our RCT described in **Chapter 5** due to slow accrual. A 6-month trial duration could not exclude a beneficial effect of lithium on long-term functional worsening. We further cannot exclude selection bias as the majority of our participants were unemployed females with significant neurocognitive impairment. Finally, all our participants were Black Xhosa speaking Africans, which limits the generalisability of our results. Our findings of no additive nephrotoxicity of lithium dosed with TFV need to be viewed in context of the study limitations.

In **Chapter 6** we reported on the safety of lithium dosed with TFV in a RCT that was not powered for this endpoint. We followed patients for 24 weeks and we can only make inferences about the short-term safety of concomitant lithium and TFV administration. We may have missed more subtle markers of tubulopathy as we did not measure urine markers of tubulopathy. Only approximately half of participants had therapeutic lithium trough concentrations. We collected lithium trough concentrations as soon as participants arrived at the study site and despite best efforts, sample collection time for some participants was beyond 12 hours. Finally, we excluded patients with renal impairment and concomitant medication which may potentiate lithium toxicity.

Impact of the studies on the field

This thesis contributes to the field of HAND research in several ways. First, we identified novel genetic associations with plasma EFV, plasma 7-OH-EFV, plasma 7-OH-EFV/EFV ratio, plasma 8-OH-EFV/EFV ratio, CSF efavirenz and CSF 8-OH-EFV/EFV ratio. We did not find pharmacokinetic-pharmacodynamic relationships between CSF EFV, 8-OH-EFV, TFV and FTC exposure and neurocognitive performance. However, we found that the *CYP2A6* -48A→C polymorphism was

independently associated with higher CSF 8-OH-EFV/EFV ratio. It is possible that EFV is metabolized into 8-OH-EFV in the CSF by the accessory pathway *CYP2A6* in *CYP2B6* slow metabolizers. Participants with detectable CSF 8-OH-EFV scored worse on the Colour Trails Test and had a higher Global Deficit Score (GDS) which was not statistically significant. It may be possible that the *CYP2A6* -48A→C polymorphism may predispose *CYP2B6* slow metabolizers to higher CSF 8-OH-EFV concentrations and worsen neurocognitive performance. Future studies should replicate our genetic associations in particular the *CYP2A6* -48A→C association with 8-OH-EFV with a larger sample size. Second, we found that lithium was not effective in the treatment of HAND in a 24-week RCT. Our study demonstrated the importance of confirmatory placebo controlled studies. Although we did not enroll our original calculated sample due to slow accrual, an increase in sample size is unlikely to change our findings as a sample size of 65 using the same assumptions as the original calculation have a power of 70% - 90%. The published GDS standard deviations on which we based our sample size were similar to the GDS standard deviation in our cohort. Future studies should study other adjunctive therapy. Third, we found that lithium can be prescribed with TFV without additive nephrotoxicity. While our data do not support the use of lithium in patients with HAND, the prevalence of bipolar disorder in HIV-infected patients is 4 to 5 times higher than the general population and the most effective mood stabiliser is lithium.[36–38] Our finding supports the renal safety of TFV-based ART in HIV-infected patients with bipolar disorder requiring lithium therapy as a mood stabiliser.

Implications for clinical practice

Our findings may have public health implications. We improved our understanding of genetic determinants of EFV-TFV-FTC exposure in the CSF which, is the current World Health Organisation recommended first-line ART.[39] EFV slow metabolizers are most prevalent in Sub-Saharan Africa and predispose to high EFV plasma and CSF

concentrations. High EFV concentrations are associated with significant morbidity and even mortality and should be monitored.[40,41]

We found a trend towards an association between neurotoxicity and higher CSF 8-OH-EFV concentrations. We confirm that there is limited clinical data to support EFV or 8-OH-EFV as a contributor to ongoing HAND irrespective of EFV metabolizer status.

Lithium does not appear to have benefit as adjunctive therapy in patients with HAND. Our findings support its use the renal safety of TFV-based ART in HIV-infected patients with bipolar disorder requiring lithium therapy as a mood stabiliser.

Future research

Further studies should follow this work. First, we may not have found a difference in GDS between lithium and placebo arms, but may detect a change in other endpoints. In our future research we will perform biomarker studies on our stored CSF and plasma samples as well as study the diffusion tremor imaging and resting-state functional magnetic resonance imaging data. Raised CSF neurofilament light chain (NFL) has been associated with ongoing axonal injury in patients with HAND and may be a marker for neuronal apoptosis, which lithium may protect against.[42] On imaging lithium induces changes in brain microstructure that may be associated with improved strength in functional connectivity.[43]

Second, adjunctive pharmacotherapy should be studied further. Paroxetine for example was associated with neuropsychological test improvement in patients with HAND in a proof-of-principle RCT and should be followed by a confirmatory trial.[44] The mechanism of the neuroprotective effect of paroxetine is unclear, but was independent of the serotonin reuptake transporter and may be via mitochondria

proteins adenine nucleotide translocase (ANT) and the voltage dependent anion channel (VDAC).[45]

Third, the genetic association of *CYP2A6* -48A→C association with 8-OH-EFV should be studied in a larger study. We should consider targeting patients who present with EFV-induced neurotoxicity.

Finally, the integrase inhibitors such as dolutegravir (DTG) are likely to replace EFV and genetic polymorphisms may be associated with DTG transfer into the CNS and associated adverse effects.[46–49] The impact of genetic polymorphisms on DTG CNS-adverse events and cognitive function in African patients are unknown and should be studied.

References

1. Heaton RK, Clifford DB, Franklin DR, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*. 2010;75(23):2087-2096. doi:10.1212/WNL.0b013e318200d727.
2. Heaton RK, Franklin DR, Ellis RJ, et al. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *J Neurovirol*. 2011;17(1):3-16. doi:10.1007/s13365-010-0006-1.
3. Joska JA, Westgarth-Taylor J, Hoare J, et al. Neuropsychological outcomes in adults commencing highly active anti-retroviral treatment in South Africa: a prospective study. *BMC Infect Dis*. 2012;12(1):39. doi:10.1186/1471-2334-12-39.
4. Cysique LA, Bain MP, Brew BJ, Murray JM. The burden of HIV-associated neurocognitive impairment in Australia and its estimates for the future. *Sex Health*. 2011;8(4):541-550. doi:10.1071/SH11003.
5. Winston A., Amin J, Clarke A., et al. Cerebrospinal Fluid Exposure of Efavirenz and Its Major Metabolites When Dosed at 400 mg and 600 mg Once Daily: A Randomized Controlled Trial. *Clin Infect Dis*. 2014:1-7. doi:10.1093/cid/ciu976.
6. Nightingale S, Chau TTH, Fisher M, et al. Efavirenz and Metabolites in Cerebrospinal Fluid: Relationship with CYP2B6 c.516G→T Genotype and Perturbed Blood-Brain Barrier Due to Tuberculous Meningitis. *Antimicrob Agents Chemother*. 2016;60(8):4511-4518. doi:10.1128/AAC.00280-16.
7. Aouri M, Barcelo C, Ternon B, et al. In vivo profiling and distribution of known and novel phase i and phase II metabolites of efavirenz in plasma, urine, and cerebrospinal fluid. *Drug Metab Dispos*. 2016;44(1):151-161. doi:10.1124/dmd.115.065839.
8. di Iulio J, Fayet A, Arab-Alameddine M, et al. In vivo analysis of efavirenz

metabolism in individuals with impaired CYP2A6 function. *Pharmacogenet Genomics*. 2009;19(4):300-309. doi:10.1097/FPC.0b013e328328d577.

9. Ngaimisi E, Habtewold A, Minzi O, et al. Importance of ethnicity, CYP2B6 and ABCB1 genotype for efavirenz pharmacokinetics and treatment outcomes: a parallel-group prospective cohort study in two sub-Saharan Africa populations. *PLoS One*. 2013;8(7):e67946. doi:10.1371/journal.pone.0067946.
10. Störmer E, von Moltke LL, Perloff MD, Greenblatt DJ. Differential modulation of P-glycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. *Pharm Res*. 2002;19(7):1038-1045. http://www.ncbi.nlm.nih.gov/pubmed/12180537.
11. Swart M, Ren Y, Smith P, Dandara C. ABCB1 4036A>G and 1236C>T Polymorphisms Affect Plasma Efavirenz Levels in South African HIV/AIDS Patients. *Front Genet*. 2012;3(NOV):1-10. doi:10.3389/fgene.2012.00236.
12. Letendre SL, Woods SP, Ellis RJ, et al. Lithium improves HIV-associated neurocognitive impairment. *AIDS*. 2006;20(14):1885-1888. doi:10.1097/01.aids.0000244208.49123.1b.
13. Schifitto G, Zhong J, Gill D, et al. Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. *J Neurovirol*. 2009;15(2):176-186. doi:10.1080/13550280902758973.
14. Benedetti F, Carlino E, Pollo A. How placebos change the patient's brain. *Neuropsychopharmacology*. 2011;36(1):339-354. doi:10.1038/npp.2010.81.
15. Murray D, Stoessl a J. Mechanisms and therapeutic implications of the placebo effect in neurological and psychiatric conditions. *Pharmacol Ther*. 2013;140(3):306-318. doi:10.1016/j.pharmthera.2013.07.009.
16. Holford N. Clinical pharmacology = disease progression + drug action. *Br J Clin Pharmacol*. 2015;79(1):18-27. doi:10.1111/bcp.12170.
17. Tedaldi EM, Minniti NL, Fischer T. HIV-Associated Neurocognitive Disorders: The Relationship of HIV Infection with Physical and Social Comorbidities.

Biomed Res Int. 2015;2015:1-13. doi:10.1155/2015/641913.

18. Hou L, Heilbronner U, Degenhardt F, et al. Genetic variants associated with response to lithium treatment in bipolar disorder: a genome-wide association study. *Lancet (London, England)*. 2016;387(10023):1085-1093. doi:10.1016/S0140-6736(16)00143-4.
19. Strakowski SM, Fleck DE, Welge J, et al. fMRI brain activation changes following treatment of a first bipolar manic episode. *Bipolar Disord*. 2016;18(6):490-501. doi:10.1111/bdi.12426.
20. Forlenza OV, Aprahamian I, de Paula VJ, Hajek T. Lithium, a Therapy for AD: Current Evidence from Clinical Trials of Neurodegenerative Disorders. *Curr Alzheimer Res*. 2016;13(8):879-886. http://www.ncbi.nlm.nih.gov/pubmed/26892289.
21. Berk M, Cowdery S, Williams L, Malhi GS. Recalibrating the risks and benefits of lithium therapy. *Br J Psychiatry*. 2017;211(1):1-2. doi:10.1192/bjp.bp.116.193789.
22. Gisslén M, Krut J, Andreasson U, et al. Amyloid and tau cerebrospinal fluid biomarkers in HIV infection. *BMC Neurol*. 2009;9:63. doi:10.1186/1471-2377-9-63.
23. Davis JM, Rosenbaum A, Shahinian V, Brosius FC. Prevention of lithium-associated renal failure: recent evidence. *The lancet Psychiatry*. 2015;2(12):1045-1047. doi:10.1016/S2215-0366(15)00498-8.
24. Shine B, McKnight RF, Leaver L, Geddes JR. Long-term effects of lithium on renal, thyroid, and parathyroid function: a retrospective analysis of laboratory data. *Lancet (London, England)*. 2015;386(9992):461-468. doi:10.1016/S0140-6736(14)61842-0.
25. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis*. 2010;51(5):496-505. doi:10.1086/655681.

26. Kohler JJ, Hosseini SH, Hoying-Brandt A, et al. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. *Lab Invest*. 2009;89(5):513-519. doi:10.1038/labinvest.2009.14.

27. Tourret J, Deray G, Isnard-Bagnis C. Tenofovir effect on the kidneys of HIV-infected patients: a double-edged sword? *J Am Soc Nephrol*. 2013;24(10):1519-1527. doi:10.1681/ASN.2012080857.

28. Kohler JJ, Hosseini SH, Green E, et al. Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. *Lab Invest*. 2011;91(6):852-858. doi:10.1038/labinvest.2011.48.

29. Kim H-R, Lee K-Y, Lee K-Y, et al. Transcriptional regulation, stabilization, and subcellular redistribution of multidrug resistance-associated protein 1 (MRP1) by glycogen synthase kinase 3αβ: novel insights on modes of cadmium-induced cell death stimulated by MRP1. *Arch Toxicol*. 2015;89(8):1271-1284. doi:10.1007/s00204-014-1381-9.

30. Clos S, Rauchhaus P, Severn A, Cochrane L, Donnan PT. Long-term effect of lithium maintenance therapy on estimated glomerular filtration rate in patients with affective disorders: a population-based cohort study. *The lancet Psychiatry*. 2015;2(12):1075-1083. doi:10.1016/S2215-0366(15)00316-8.

31. Haas DW, Kwara A, Richardson DM, et al. Secondary metabolism pathway polymorphisms and plasma efavirenz concentrations in HIV-infected adults with CYP2B6 slow metabolizer genotypes. *J Antimicrob Chemother*. 2014;69(8):2175-2182. doi:10.1093/jac/dku110.

32. Holzinger ER, Grady B, Ritchie MD, et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. *Pharmacogenet Genomics*. 2012;22(12):858-867. doi:10.1097/FPC.0b013e32835a450b.

33. Avery LB, Sacktor N, McArthur JC, Hendrix CW. Protein-free efavirenz concentrations in cerebrospinal fluid and blood plasma are equivalent:

applying the law of mass action to predict protein-free drug concentration. *Antimicrob Agents Chemother*. 2013;57(3):1409-1414. doi:10.1128/AAC.02329-12.

34. Yilmaz A, Price RW, Gisslén M. Antiretroviral drug treatment of CNS HIV-1 infection. *J Antimicrob Chemother*. 2012;67(2):299-311. doi:10.1093/jac/dkr492.
35. Van de Wijer L, Schellekens AFA, Burger DM, Homberg JR, de Mast Q, van der Ven AJAM. Rethinking the risk-benefit ratio of efavirenz in HIV-infected children. *Lancet Infect Dis*. 2016;16(5):e76-e81. doi:10.1016/S1473-3099(16)00117-1.
36. Moore DJ, Posada C, Parikh M, et al. HIV-Infected Individuals with Co-occurring Bipolar Disorder Evidence Poor Antiretroviral and Psychiatric Medication Adherence. *AIDS Behav*. 2012;16(8):2257-2266. doi:10.1007/s10461-011-0072-2.
37. de Sousa Gurgel W, da Silva Carneiro AH, Barreto Rebouças D, et al. Prevalence of bipolar disorder in a HIV-infected outpatient population. *AIDS Care*. 2013;25(12):1499-1503. doi:10.1080/09540121.2013.779625.
38. Miura T, Noma H, Furukawa TA, et al. Comparative efficacy and tolerability of pharmacological treatments in the maintenance treatment of bipolar disorder: a systematic review and network meta-analysis. *The Lancet Psychiatry*. 2014;1(5):351-359. doi:10.1016/S2215-0366(14)70314-1.
39. Organisation WH. First-line ART for Adults. http://www.who.int/hiv/pub/guidelines/arv2013/art/artadults/en/. Accessed June 1, 2017.
40. Kenyon C, Mfolozi S, Croxford R, Colebunders R, Cohen K. Severe efavirenz-induced vacuolar axonopathy complicated by fatal aspiration pneumonia. *Br J Clin Pharmacol*. 2012;74(6):1070-1072. doi:10.1111/j.1365-2125.2012.04299.x.

41. Variava E, Sigauke FR, Norman J, et al. Brief Report: Late Efavirenz-Induced Ataxia and Encephalopathy: A Case Series. *J Acquir Immune Defic Syndr*. 2017;75(5):577-579. doi:10.1097/QAI.0000000000001451.

42. Jessen Krut J, Mellberg T, Price RW, et al. Biomarker evidence of axonal injury in neuroasymptomatic HIV-1 patients. *PLoS One*. 2014;9(2):e88591. doi:10.1371/journal.pone.0088591.

43. Tivarus ME, Pester B, Schmidt C, et al. Are Structural Changes Induced by Lithium in the HIV Brain Accompanied by Changes in Functional Connectivity? *PLoS One*. 2015;10(10):e0139118. doi:10.1371/journal.pone.0139118.

44. Sacktor N, Skolasky RL, Moxley R, et al. Paroxetine and fluconazole therapy for HIV-associated neurocognitive impairment: results from a double-blind, placebo-controlled trial. *J Neurovirol*. 2018;24(1):16-27. doi:10.1007/s13365-017-0587-z.

45. Steiner JP, Bachani M, Wolfson-Stofko B, et al. Interaction of paroxetine with mitochondrial proteins mediates neuroprotection. *Neurotherapeutics*. 2015;12(1):200-216. doi:10.1007/s13311-014-0315-9.

46. Kheloufi F, Allemand J, Mokhtari S, Default A. Psychiatric disorders after starting dolutegravir: report of four cases. *AIDS*. 2015;29(13):1723-1725. doi:10.1097/QAD.0000000000000789.

47. Scheper H, van Holten N, Hovens J, de Boer M. Severe depression as a neuropsychiatric side effect induced by dolutegravir. *HIV Med*. 2017:1-2. doi:10.1111/hiv.12538.

48. Chen S, St Jean P, Borland J, et al. Evaluation of the effect of UGT1A1 polymorphisms on dolutegravir pharmacokinetics. *Pharmacogenomics*. 2014;15(1):9-16. doi:10.2217/pgs.13.190.

49. Yagura H, Watanabe D, Kushida H, et al. Impact of UGT1A1 gene polymorphisms on plasma dolutegravir trough concentrations and neuropsychiatric adverse events in Japanese individuals infected with HIV-1. *BMC Infect Dis*. 2017;17(1):622. doi:10.1186/s12879-017-2717-x.